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THE TRANSFUSION EXPERIMENT WITH RED BLOOD CORPUSCLES

HISANOBU KAMBE AND ETSUZO KOMIYA

From the Medical Department, the Imperial University, Tokyo, Japan

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What is the fate of red blood corpuscles if they have been introduced into an animal body of same species? This problem has been studied by numerous investigators (1), (2), (3) and though there are still objections it is generally believed that the transfused red cells are capable of functioning in the animal body. Whether these red cells remain and function in circulation of a recipient animal or whether they are decomposed, was formerly studied chiefly by means of the examination of the changes of urine or the bodily condition of the animal. Recent opinion indicates that the most satisfactory method of attacking the problem as to the length of life of the transfused corpuscles is to study the changes in erythrocyte count following transfusion.

In the earlier work in which this method was used little attention was given to morphological changes in the corpuscles. This is a point of considerable importance because from the erythrocyte count alone we are not justified in drawing conclusions as to the biological phenomena exhibited by the foreign corpuscle or its host. It is therefore desirable to collect more data on the subject.

EXPERIMENTAL

In the present experiments full-grown rabbits were used. To obtain blood from a donor the carotid artery was exposed by usual method and opened on one side with a sterile scalpel. The blood thus collected in a sterile, thick-walled flask containing glass beads, was defibrinated by vigorous shaking, filtered into another sterile flask through double gauze, and preserved for injection. Sometimes blood was kept in a mixture of isotonic citrate and dextrose solution, and when this mixture was used the supernatant fluid was pipetted away.

To render a recipient animal anemic, blood q. l. was withdrawn from carotid artery without anesthesia, care being taken to avoid the shock which may be caused by a rapid hemorrhage. The blood obtained from the donor was then transfused into the ear vein of recipient rabbits using a sterile syringe. At the time of injection the blood was warmed to the body temperature. In each series of experiments, the number of red cells, hemoglobin content and morphology of the blood were carefully studied. The number of red cells was counted by the Thoma-Zeiss apparatus, and hemoglobin content was estimated by Sahli's hemometer. Blood cells were stained in May-Grünwald-Giemsa's solution. Each experiment was repeated several times with almost the same result. To avoid a repetition of data only one instance for each case is recorded here.

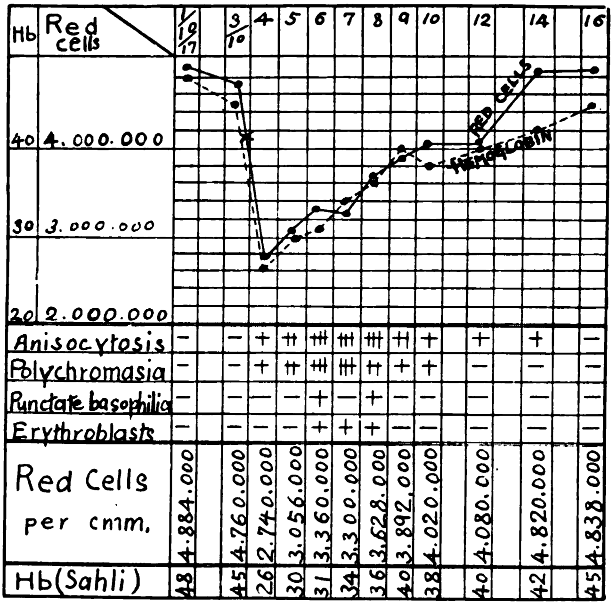
Control experiment. In the first place an experiment was done to observe in what manner the natural restoration of the anemia caused by hemorrhage takes place and what changes in the direction of morphology are brought about.

Experiment 1. Rabbit weighing 2500 grams was bled 65 cc. from the carotid artery. The animal has been always in a good condition.

It may be seen from figure 1 that if the blood of 20–30 cc. per kilogram of body weight is depleted, the number of red cells of the animal is generally reduced by 40 per cent or more of its original amount and with a gradual improvement it will return to normal level two weeks later.

As to morphological changes, polychromatophilia was especially noticeable. This kind of red cells appeared in twelve or twenty-four hours after hemorrhage, and gradually increasing in number they reached the highest point on the third or fourth day, disappearing again in the course of a week. These polychromatic red cells are larger than normal red cells, so the blood has appearance of a remarkable anisocytosis. Erythroblasts and basophilic punctates may also be seen more or less in the early stages of anemia, but they are rather inconstant or few in number. These results, as is well known, indicate an abnormal activity on the part of the bone-marrow in the sense of compensation for a loss of blood cells by hemorrhage.

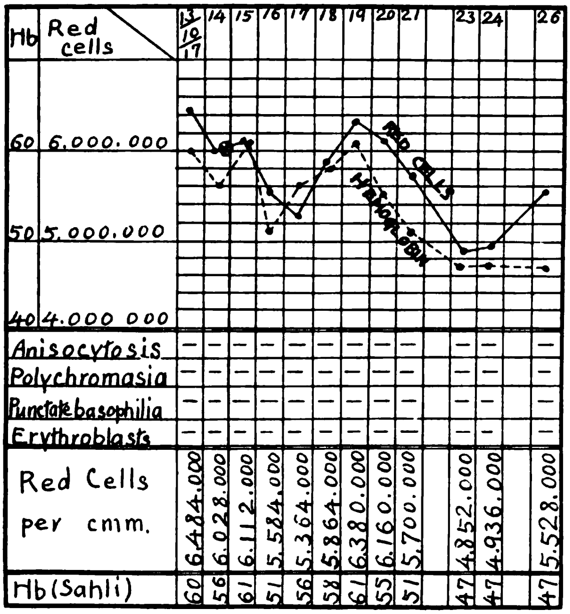
Transfusion experiment. From the above recorded fact it is easily expected that if transfused red blood corpuscles may function in the



Hb / Red cells	1/10/17	3/10	4	5	6	7	8	9	10	12	14	16
Anisocytosis	−	−	+	++	+++	+++	+++	++	+	+	+	−
Polychromasia	−	−	+	++	+++	+++	++	+	+	−	−	−
Punctate basophilia	−	−	−	−	+	−	+	−	−	−	−	−
Erythroblasts	−	−	−	−	+	+	+	−	−	−	−	−
Red Cells per cmm.	4.884.000	4.760.000	2.740.000	3.056.000	3.360.000	3.300.000	3.628.000	3.892.000	4.020.000	4.080.000	4.820.000	4.838.000
Hb (Sahli)	48	45	26	30	31	34	36	40	38	40	42	45

Fig. 1

* = bleeding; o = transfusion.

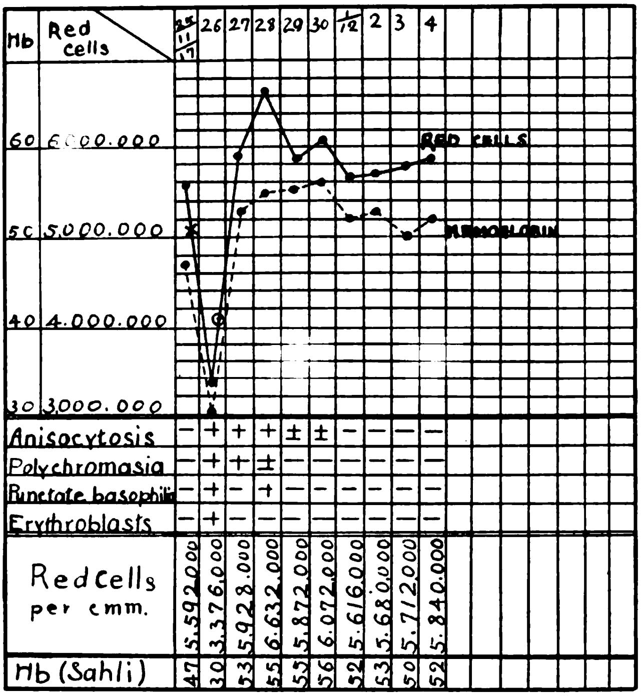


Hb / Red cells	13/10/17	14	15	16	17	18	19	20	21	23	24	26
Anisocytosis	−	−	−	−	−	−	−	−	−	−	−	−
Polychromasia	−	−	−	−	−	−	−	−	−	−	−	−
Punctate basophilia	−	−	−	−	−	−	−	−	−	−	−	−
Erythroblasts	−	−	−	−	−	−	−	−	−	−	−	−
Red Cells per cmm.	6.484.000	6.028.000	6.112.000	5.584.000	5.364.000	5.864.000	6.380.000	6.160.000	5.700.000	4.852.000	4.936.000	5.528.000
Hb (Sahli)	60	56	61	51	56	58	61	55	51	47	47	47

Fig. 2

animal body and an amount of blood is enough to make up for the previous deficit and if replacement is done as soon as the animal has been bled, there will not occur any change in the blood picture, neither numerically nor morphologically, the bone-marrow being subjected to no anemic stimulation, while in the reverse case such changes will take place.

In general we have noted the volume, the erythrocyte count and the hemoglobin of the drawn blood, and have calculated from these the amount of cell suspension of known hemoglobin content that should be injected to restore the animal's hemoglobin content to the normal.



	25/11/17	26	27	28	29	30	1/12	2	3	4
Anisocytosis	−	+	+	+	±	±	−	−	−	−
Polychromasia	−	+	+	±	−	−	−	−	−	−
Punctate basophili	−	+	−	+	−	−	−	−	−	−
Erythroblasts	−	+	−	−	−	−	−	−	−	−
Red Cells per cmm.	5.592.000	3.376.000	5.928.000	6.632.000	5.872.000	6.072.000	5.616.000	5.680.000	5.712.000	5.840.000
Hb (Sahli)	47	30	53	55	55	56	52	53	50	52

Fig. 3

Experiment 2. Rabbit weighing 2000 grams was bled (40 cc.) from the carotid artery, and then 50 cc. of defibrinated fresh blood were injected at once. The animal was very lively.

In the case in which transfusion was done immediately after bleeding, neither hemoglobin content nor number of red cells indicates any change, as figure 2 shows; morphological figures are entirely the same with the normal.

Experiment 3. The used rabbit weighed 3000 grams. Seventy-six cubic centimeters of blood were taken and after about 20 hours the same amount of defibrinated fresh blood was injected.

In the case in which blood has been replaced within twelve or twenty-four hours after bleeding, the hemoglobin content as well as the red cell count returned to normal level simultaneously with the injection, while the disappearance of polychromatophilia and anisocytosis took place after a few days, but in this case the degree of morphological change was milder and the duration of its appearance shorter than those of the control experiment (fig. 3). This fact illustrates that the bone-marrow was subjected to a transitory stimulation, a certain time having elapsed before injection.

As is evident from the results of the above experiments, the transfused red blood corpuscles are capable of functioning.

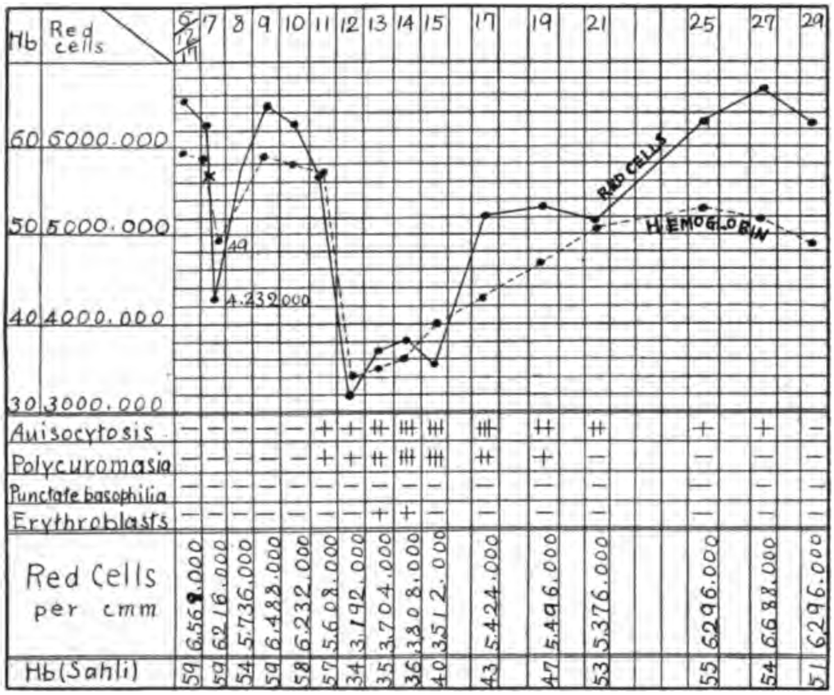
Among these latter experiments we met with two very interesting instances, that is, the animal dealt with as just described developed a sudden anemia on the fourth day after the blood pictures had once returned to normal. Furthermore polychromatophilia and anisocytosis in a large number, corresponding to the number of red cells, appeared again in circulation. This anemia returned to normal condition in the course of fourteen days as in the case of the experiment 1 (fig. 4).

Experiment 4. Rabbit of body weight of 2750 grams was bled 60 cc. at one time from the carotid artery. After four hours 65 cc. of defibrinated blood kept in ice for forty-eight hours were introduced. Animal remained in very excellent condition, very lively.

These results indicate evidently that the transfused red cells were destroyed on the fourth day after transfusion. We shall refer to the possible reason for this later.

Our study will turn now to determine how long these red cells are kept alive from the view of their capacity of functioning in the animal body. Landois, who has studied this problem extensively, states that cells kept for two days at 5°C. exert no harmful effect on the animal, but those kept for three days give rise to albuminuria and hematuria and cause death on the second day after transfusion. In 1916 Rous and Turner (4) developed a method for preserving living red blood cells in vitro and determined the length of their life by means of transfusion (5). This is the only report which paid attention to the morphology, so far as we are able to find in the literature.

They found that erythrocytes preserved in mixture of blood, sodium citrate and water for fourteen days remained in circulation and functioned so well that the animal showed no disturbance, and the blood count, hemoglobin and percentage of reticulated cells remained unvaried, while cells kept for twenty-three days, though apparently intact and unchanged when transfused, soon left the circulation.

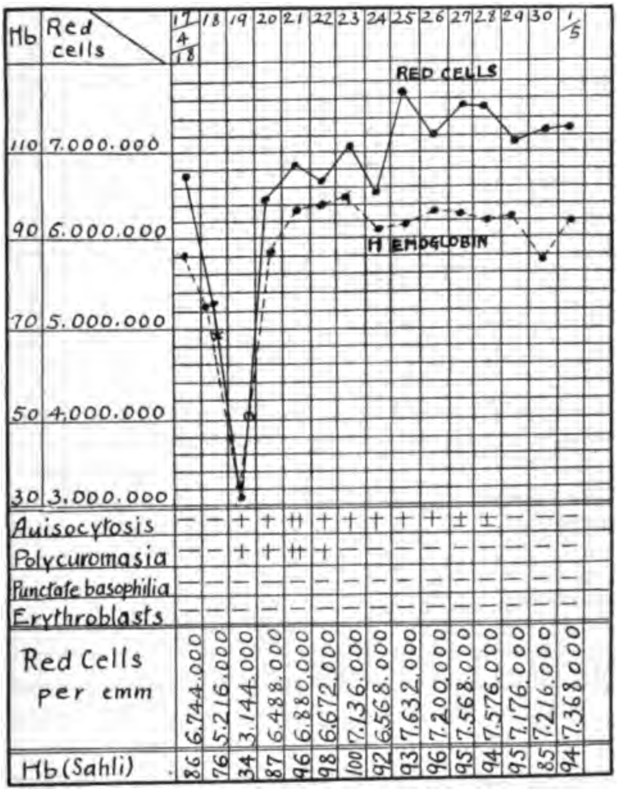


	6/12/19	7	8	9	10	11	12	13	14	15		17		19		21				25		27		29
Auisocytosis	−	−	−	−	−	+	+	++	+++	+++		+++		++		++				+		+		−
Polycuromasia	−	−	−	−	−	+	+	++	+++	+++		++		+		−				−		−		−
Punctate basophilia	−	−	−	−	−	−	−	−	−	−		−		−		−				−		−		−
Erythroblasts	−	−	−	−	−	−	−	+	+	−		−		−		−				−		−		−
Red Cells per cmm	6.569.000	6.216.000	5.736.000	6.488.000	6.232.000	5.608.000	3.192.000	3.704.000	3.808.000	3.512.000		5.424.000		5.496.000		5.376.000				6.296.000		6.688.000		6.296.000
Hb (Sahli)	59	59	54	59	58	57	34	35	36	40		43		47		53				55		54		51

Fig. 4

Our experiments were made with defibrinated blood and blood-citrate-dextrose mixture to determine the availability for functional use of red cells kept in vitro.

Experiment with defibrinated blood. When sterile blood was put at once on ice it showed no trace of hemolysis in the supernatant fluid after ten days, while on the twentieth day of preservation a very slight hemolysis was seen, but the number of red cells did not show a marked decrease, and the morphological character of the cells seemed entirely normal. When blood was kept for thirty days, there was some hemolysis and also an appreciable decrease in number of red cells. In the fresh and stained preparations the blood appeared normal. In experiments 5, 6, 7 and 8 the blood thus preserved for various periods was transfused.



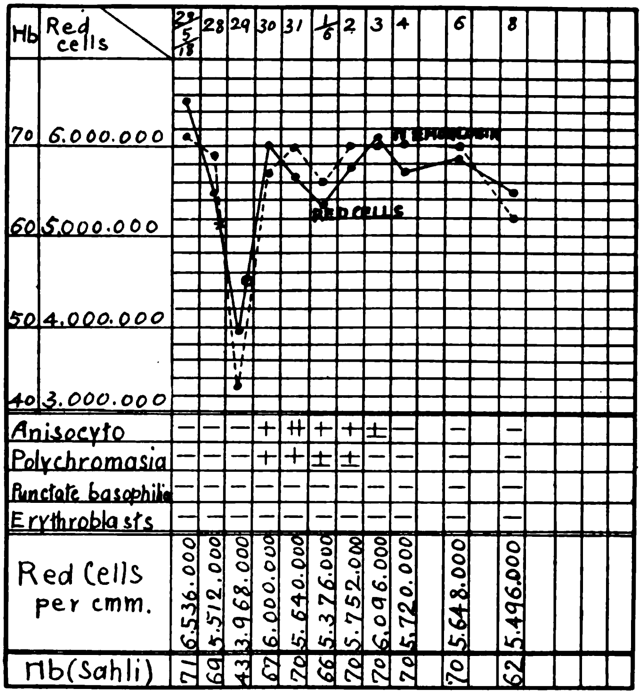
	17/4/18	18	19	20	21	22	23	24	25	26	27	28	29	30	1/5
Auisocytosis	–	–	+	+	++	+	+	+	+	+	±	±	–	–	–
Polycuromasia	–	–	+	+	++	+	–	–	–	–	–	–	–	–	–
Punctate basophilia	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Erythroblasts	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Red Cells per cmm	6.744.000	5.216.000	3.144.000	6.488.000	6.880.000	6.672.000	7.136.000	6.568.000	7.632.000	7.200.000	7.568.000	7.576.000	7.176.000	7.216.000	7.368.000
Hb (Sahli)	86	76	34	87	96	98	100	92	93	96	95	94	95	85	94

Fig. 5

Experiment 5. Rabbit weighing 2300 grams was bled twice (55 and 35 cc.). After twenty-four hours the equivalent amount (hemoglobin content 90 per cent) of defibrinated blood kept for three days was injected.

Experiment 6. Rabbit weighing 2050 grams was bled (40 cc.) and twenty-four hours later 38 cc. of blood which had been taken eleven days previously were injected (number of cells in transfusate 5,456,000 in c.mm.).

Experiment 7. Body weight: 2100 grams. The recipient rabbit was bled (50 cc.) and at once the same amount of blood preserved for twenty days was transfused. The animal manifested at no time symptoms of distress.



	27/5/18	28	29	30	31	1/6	2	3	4		6		8				
Anisocyto	—	—	—	+	++	+	+	±	—		—		—				
Polychromasia	—	—	—	+	+	±	±	—	—		—		—				
Punctate basophilia	—	—	—	—	—	—	—	—	—		—		—				
Erythroblasts	—	—	—	—	—	—	—	—	—		—		—				
Red Cells per cmm.	6.536.000	5.512.000	3.968.000	6.000.000	5.640.000	5.376.000	5.752.000	6.096.000	5.720.000		5.648.000		5.496.000				
Hb(Sahli)	71	69	43	67	70	66	70	70	70		70		62				

FIG. 6

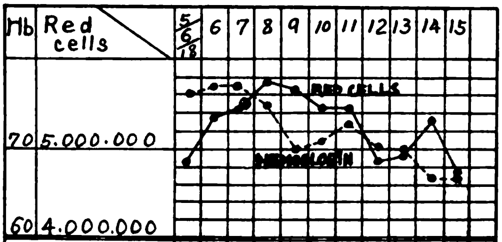
As shown in charts 5, 6 and 7, red blood cells in defibrinated blood are capable of functioning even when they have been kept in vitro for three weeks.

Experiment 8. Experiments were all done with cells kept for thirty days in vitro.

A. An animal weighing 3150 grams was bled twice (70 cc. and 30 cc.), and an equivalent amount of kept cells was injected. The rabbit lived for two days but neither the number of red cells nor the hemoglobin content returned to normal. In morphological figures there appeared noteworthy alterations corresponding to this change (fig. 8, *A*).

B. Body weight 1900 grams. The animal was bled 38 cc. and then 50 cc. of blood (cell count in c.mm.: 3,112,000) were introduced. This rabbit was alive for a long time after the operation, without showing any increase in number of red cells and hemoglobin percentage in consequence of transfusion. Thus in natural recovery of above mentioned anemia, they returned to normal in the course of ten days. At this time an appreciable change in morphology was noted.

Two other animals died within one-half or one hour after transfusion.



	5/6/18	6	7	8	9	10	11	12	13	14	15	
Anisocytosis	–	–	–	–	–	–	–	–	–	–	–	
Polychromasia	–	–	–	–	–	–	–	–	–	–	–	
Punctate basophilia	–	–	–	–	–	–	–	–	–	–	–	
Erythroblasts	–	–	–	–	–	–	–	–	–	–	–	
Red Cells per cmm	4.696.000	5.392.000	5.424.000	5.752.000	5.704.000	5.496.000	5.448.000	4.840.000	4.936.000	5.376.000	4.592.000	
Hb (Sahli)	76	77	77	75	70	71	73	70	70	67	67	

Fig. 7.

Both animals contained pitch-dark colored hemoglobinuria in the bladders.

Transfusion with cells kept in a blood-citrate-dextrose mixture. For the purpose of preservation three parts of the blood were mixed with five parts of isotonic citrate and two parts of isotonic dextrose solution. When they were to be used the supernatant fluid was pipetted off, the cells suspended in 0.85 per cent sodium chlorid solution until the original quantity of blood was reached and then used for injection. The mixture could be kept for one month without showing any trace of hemolysis. On the thirtieth day there began to appear a very slight hemolysis.

Experiment 9. Rabbit weighing 2500 grams was bled (50 cc.) and replaced after twenty-four hours with 60 cc. blood (number of blood corpuscles 4,516,000 in c.mm.) which had been kept for thirty days.

As figure 9 illustrates, the result was entirely similar to those cases in which fresh blood was transfused.

Experiment 10. A rabbit weighing 2850 grams was used. Two bleedings (60 cc. and 35 cc.) were effected; after having left the animal in anemic condition for forty-eight hours, there appeared a large number of young cell forms in the circulation. After transfusion with an equivalent amount of blood cells preserved in ice for forty days, these cells disappeared within a few days from the circulation, indicating that the transfused blood corpuscles function entirely normally (fig. 10).

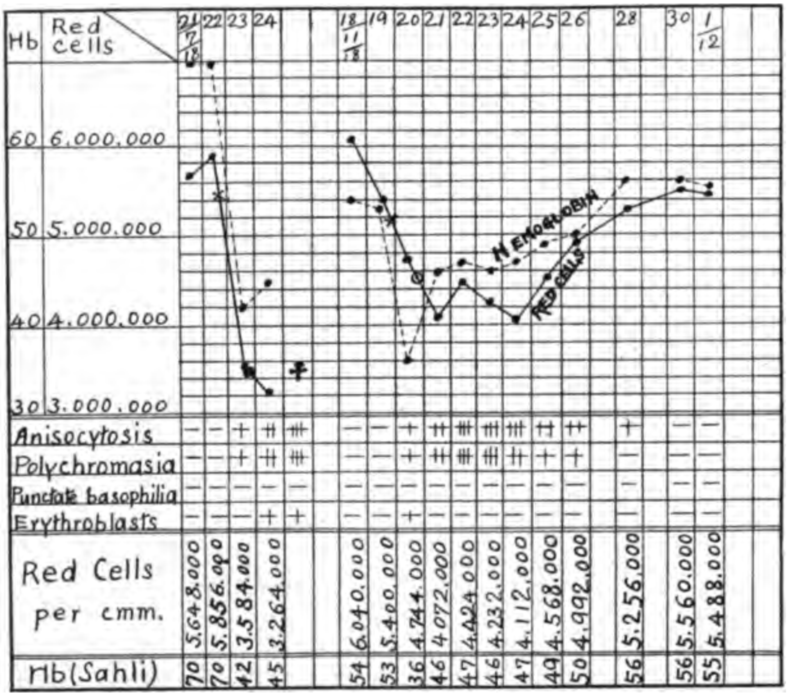


Fig. 8

In this experiment it happened that the red cell count showed a gradual decrease from the fifth day after transfusion, reaching its maximum drop on the ninth day, after which a gradual recovery ensued. Morphological figures ran parallel to the changes in number of red blood corpuscles. Marked polychromatophilia and anisocytosis, etc., which once had been restored to normal, appeared again on the tenth day, but at the time of recovery from anemia had disappeared altogether.

The experiments so far have shown that red cells may preserve their vitality in vitro for a long period, namely, in defibrinated blood for twenty days, in the mixture of blood-citrate-dextrose even for thirty days or more, and may function normally in the animal body when transfused. Our results therefore differ somewhat from the findings of Rous and Turner who concluded that cells kept for twenty-three days in their preservative mixture, though apparently intact and unchanged, soon left the circulation.

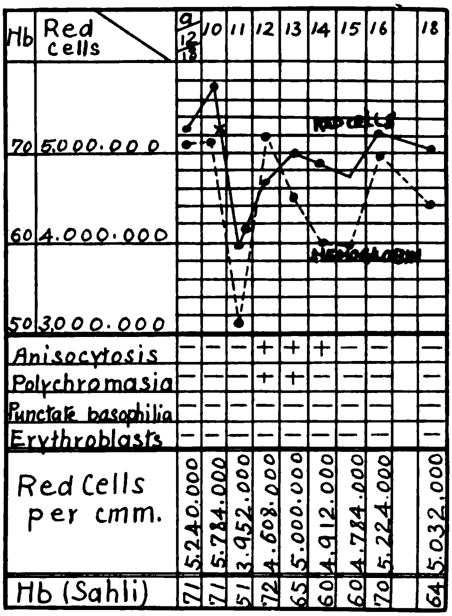
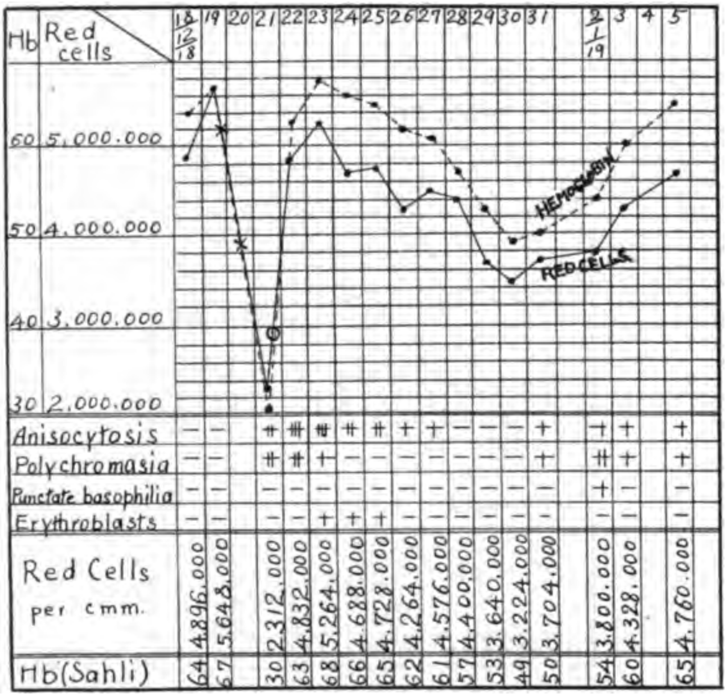


Fig. 9

Preceding the conclusion of this work we will touch briefly on the cause of the peculiar cases of acute anemia above mentioned (exper. 4), which have developed at a certain period after transfusion. At first we assumed that this anemia may occur due to the destruction of red cells, being caused by the injurious effect on the function by cold, because such transfusion experiments with blood preserved in ice showed in succession the same results, having initiated a sudden drop of hemoglobin on the fourth day after transfusion. But it became evident afterwards, as a number of experiments proved, that this destruction of red cells is not simply due to the preservation or the refrigeration by ice. Rous and Robertson (6) made a report similar to ours, having injected 10 cc. of blood every other day. They stated that in several animals in which the agglutinin was strongest, the plethora was suddenly succeeded by severe anemia, despite continued transfusion.



Hb / Red cells	18/12/18	19	20	21	22	23	24	25	26	27	28	29	30	31		2/1/19	3	4	5	
Anisocytosis	–	–		++	+++	+++	++	++	+	+	–	–	–	+		+	+		+	
Polychromasia	–	–		++	++	+	–	–	–	–	–	–	–	+		++	+		+	
Punctate basophilia	–	–		–	–	–	–	–	–	–	–	–	–	–		+	–		–	
Erythroblasts	–	–		–	–	+	+	+	–	–	–	–	–	–		–	–		–	
Red Cells per cmm.	4.896.000	5.648.000		2.312.000	4.832.000	5.264.000	4.688.000	4.728.000	4.264.000	4.576.000	4.400.000	3.640.000	3.224.000	3.704.000		3.800.000	4.328.000		4.760.000	
Hb (Sahli)	64	67		30	63	68	66	65	62	61	57	53	49	50		54	60		65	

Fig. 10.

Not having made any serological researches, we hesitate to assert whether or not in our cases such strong agglutinin was also existing. We could, however, demonstrate afterwards occasionally in a number of transfusion experiments such hemoagglutinin, though not so strong, similar to that pointed out by the above authors with regard to the character of temperature control of the agglutination, the persistence of the agglutinating principle and many other points. Among those cases we had one instance (exper. 10) which developed anemia on the fifth day after transfusion, but more slowly than the acute cases above stated, reaching its maximum in the course of four days.

The plasma of this rabbit agglutinated not only the cells of its own, but also those of the same species and showed this activity even when diluted 150 times; it was also disposed to have stronger activity, in spite of a gradual recovery of anemia.

Rous and Robertson reported:

> We have chilled, without result, two plethoric rabbits possessing a weak agglutinin in the hope of initiating a drop in the hemoglobin. The chilling was accomplished by means of ice-cold water, in which the well shaved ear of the rabbit was submerged for ½ or 1 hour. Throughout this period the circulation in the cold ear was exceptionally good. The rectal temperature fell to 37°C., considerably below the normal for the rabbit, but not enough to produce the in vitro agglutination of blood corpuscles.

It seems probable that the agglutinin above described including also the case of Rous has no direct causal relation to such anemia and the question arises whether an uncommon hemolysin may play perhaps an important rôle.

Furthermore the fact that such occurrence has been noticed only in the experiments performed in cold winter time and not in other seasons, leads us to believe that it may have some connection with paroxysmal hemoglobinuria.

SUMMARY

1. Transfused red cells even preserved in ice for a long time, not to mention fresh blood, are capable of functioning if transfused into the animal body of same species.
2. Erythrocytes preserved as defibrinated blood maintain their normal vitality for twenty days, in the mixtures of isotonic sodium citrate and isotonic dextrose, even for thirty days and more; thus they may be used to replace the blood lost.
3. A sudden anemia occasionally develops a few days after transfusion. There is a possibility that this is due to the isolysin, even though it occurred only in isolated cases. We are, however, unable to draw a sure conclusion from the present work, but will pursue further our studies which may throw some light on this point.

The authors desire to express their thanks to Prof. Dr. T. Irisawa for his suggestions and encouragement throughout the course of this work; and also to Prof. S. Mita for his kind advice.

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ON THE REGENERATION OF THE VAGUS NERVE

F. T. ROGERS

From the Hull Physiological Laboratory, University of Chicago

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Three years ago, in connection with work on nerve crossing between the phrenic and vagus, attention was again directed to the doubt existing as to the possibility of functional return after section and suture of the vagus. This, according to Langley (1) and Tuckett (2), and more recently by Schaffer (3) is doubtful or, if it occurs, it does so only after the lapse of years. As a control on other work it was decided to repeat the old experiment of section and suture of one vagus leaving the other intact and after as long a time for recovery as practicable in the laboratory, test the nerve for functional activity. In order to avoid the uncertainties involved in testing functional regeneration by electric stimulation, resort was made to the old suggestion of subsequently cutting the intact vagus leaving the regenerating nerve to exert such action as it might. This conclusion was forced by the negative results following stimulation of the nerve when only a few months were allowed for regeneration. The results of this series of experiments confirmed the similar experiments made by others using the same method (1), (4).

The first series of experiments consisted of four cats and one dog in each of which one vagus and cervical sympathetic were sectioned and sutured just below the level of the thyroid gland. After time intervals of from three weeks to six months the nerve was tested electrically with the animal under ether anesthesia and with arterial pressure recorded from the carotid artery (table 1). In every case it was found that while stimulation of the normal nerve produced the usual cardiac inhibition and fall in blood pressure, the stimulation of the previously sectioned and sutured nerve caused no cardiac inhibition and no gastric motor effects when stimulated on either side of the point of suture. Stimulation of this trunk above and below the scar did cause the usual respiratory inhibition and gave reflex effects on the blood pressure. With reference to the actual recovery of efferent fibers, Tuckett states that after three years this does occur. The only other similar positive statement that I have found is that of a single observation of Stewart (5), of which he states that "some regeneration appeared to have taken place (after three hundred days) since stimulation of the nerve caused slowing and weakening of the heart."

TABLE 1

In each animal of this series one vagus was sectioned and sutured, except in the case of cat 17 in which the nerve was crushed by hemostatic forceps and not sectioned. After the time intervals indicated in the table, the animals were etherized, carotid blood pressure tracings made and both the normal and the previously sectioned vagi were stimulated with tetanizing current. Stimulation was applied above and below the scar of union, but in no case did the effects differ. In all the animals of this series it was found at autopsy that the two ends of the nerve were united by a small neuroma

ANIMAL	TIME ALLOWED FOR REGENERATION	EFFECTS OF STIMULATING THE SUTURED VAGUS			
		Heart	Respiration	Blood pressure	Stomach
	months				
Cat 28	2	No inhibition	Inhibition		
Cat 12	4	No inhibition	Inhibition		No contractions
Cat 18	5	No inhibition	Inhibition	Weak stimulation pressor effect, strong stimulation depressor effect	
Cat 17	6	No inhibition	Inhibition	Depressor effect	
Dog 40	4	No inhibition	Inhibition		No contractions

Of the second set of animals only two dogs survived the long time set for the experiment (tables 2 and 3.) In two dogs, nos. 95 and 96, twenty and sixteen months respectively elapsed between the section of the one nerve and the section of the remaining vagus. In one case the animal lived thirty-four days and in the other sixteen days after cutting the second nerve. The cause of death in both cases seemed to be starvation due to paralysis of the esophagus and continued vomiting which followed attempts to eat. Respiration continued at a normal rate and amplitude during this interval of life, save that at times both dogs showed a hiccough-like disorder associated with cough that seemed to be due to irritation of the respiratory tract by vomited material. This continuation of normal breathing might be considered confirmatory of Schaffer's recent findings that section of the vagi in the cat causes little change in the respiratory rhythm provided asphyxia be prevented by keeping the larynx open. The fact noted in the tabulated findings that in one of my animals the section of the regenerated nerve after the previous section of the other vagus was followed by a slowing and deepening of the breathing, indicates regeneration had occurred, of either or both, the afferent pulmonary fibers or motor fibers to the laryngeal muscles. According to Vanlair (6), functional regeneration of the motor fibers of the recurrent laryngeal nerve can be demonstrated after one year. The facts just stated above seem to confirm this finding of Vanlair but unfortunately the writer was unable in this dog to make any observation as to the part played by the larynx in this change of respiration.

With reference to the heart an interesting condition was found Electrical stimulation of the regenerating vagus, with the animal under ether anesthesia, caused no cardiac inhibition. Sectioning of the normal nerve so as to leave only the regenerating nerve in relation to the heart, was followed by a marked increase in the rate of the heart beat. These facts of negative results to electric stimulation and an immediate increase of the heart rate after cutting the remaining normal nerve indicate that the regenerating nerve was not functional for it is common knowledge that section of only one vagus leads to only a slight cardiac acceleration. This conclusion was subsequently confirmed by cutting the regenerating nerve which caused no change in the heart rate (table 2).

In spite of these indications of absence of function in the regenerating fibers, the rate of the heart beat daily became less and in two weeks the rate was that of a normal animal. In other words, with one vagus degenerated and the other not functional, the cardiac rhythm returned to a normal rate. This fact was also noted by Stewart.

When this stage of recovery had been reached, the injection of atropine gave a tremendous increase in the heart rate. This effect was evidently due to some other factor than that of paralysis of vagus fibers. The writer hesitates to speculate on the mechanism of this atropine effect. It recalls the observation of Carlson (7) that atropine stimulates the heart ganglion of Limulus and suggests that the usual effect of atropine in the normal animal is twofold, paralyzing the extrinsic inhibitors and stimulating the automatic nerve mechanism.

In dog 95 a fortunate incident gave direct ocular proof of the fact that the vagus inhibitory fibers to the heart can regenerate. As stated

TABLE 2

Dog 96

July 4, 1918. Section and suture of the right vago-sympathetic. The dog is three and a half months of age
October 15, 1919. Gastric fistula is made

DATE	HOUR	PROCEEDING	RESPIRATION	HEART RATE	REMARKS
Nov. 1	2:00 p.m.	Dog quiet	12 per min.	84	
Nov. 1	5:00 p.m.	Left vagus sectioned			
Nov. 1	10:00 p.m.		12 per min.	230	Vomiting during the night
Nov. 2	11:00 a.m.		20 per min.	162	
Nov. 2	9:00 p.m.		9-12 per min. irregular		
Nov. 3			14 per min. irregular	130	Eating without vomiting
Nov. 4			13 per min. irregular	142	Eating and vomiting
Nov. 5			8 per min. labored	142	Vomiting and cough
Nov. 6			9 easier	146	Alert, wags tail. Vomits and coughs
Nov. 7			10 per min.	136	Eats nothing
Nov. 11	3:00 p.m.	Stomach tracing made	10 per min.	116	Getting very thin
Nov. 11	4:30 p.m.	0.3 cc. of 0.1 per cent atropine			
Nov. 11	5:00 p.m.			149	
Nov. 12	1:00 p.m.		10 per min.	114	Very thin, eats nothing
Nov. 12	5:00 p.m.	Cut the right vagus			
Nov. 12	11:00 p.m.		5 per min.	112	
Nov. 14	2:00 p.m.		6 per min.	92	
Nov. 14	3:00 p.m.	0.3 cc. atropine 0.1 per cent			
Nov. 14	3:15 p.m.		6 per min.	160	
Nov. 14	6:00 p.m.		6 per min.	122	

Nov. 16, 2:00 p.m. Dog dead. Lungs, hyperemic patches with two pus pockets, ½ inch diam. Scraps of food in stomach. The two ends of the vagi united.

Observations at time of cutting the second vagus, November 1, 1920.

Blood pressure recorded from the left carotid artery.

Balloon in stomach connected with water manometer, to record gastric contractions and respiration.

Dog under ether anesthesia: stimulation with tetanizing current.

1. Effects of stimulating left vagus before the nerve was cut.

Heart, inhibition and fall of carotid pressure (fig. 1, A). Respiration, inhibition.

Stomach, strong contraction followed by weaker ones (fig. 1, A).

2. Effects of stimulating the right nerve which had been cut and sutured.

Stimulation central to the scar of union.

Heart, no inhibition. Rise in blood pressure.

Stomach, no contraction.

Respiration, inhibited.

Stimulation peripheral to scar of union.

Heart, no change. Rise in blood pressure (fig. 1, B).

Stomach, no contraction (fig. 1, B).

Respiration, inhibited.

3. Repeat the stimulation of the normal nerve.

Normal effects on heart and stomach, as in paragraph 1 above.

4. Cut the left vagus and closed the wound in the neck.

previously, the dogs seemed to die of starvation. Two or three days before death the animals became inactive and went into a comatose condition. In the case of this animal, I chanced to find him when death was imminent. The dog was cold to touch, breathing was barely preceptible at three or four times per minute. Since the animal was dying, it was killed by opening the thorax without anesthesia. The heart was beating slowly at the rate of 40 per minute. The regenerating vagus trunk was stimulated by tetanizing current, twice above and twice below the scar marking the point of suture. Each time of stimulation the heart ceased beating for five to ten seconds and resumed beating at a slower rate than that preceding the stimulation. Unless there be accessory cardiac fibers outside the main vago-sympathetic trunk, which had escaped section, this observation indicates that regeneration of the vago-inhibitory fibers may occur if sufficient time be allowed. And it lends force to the criticism that electric stimulation of regenerating nerves in anesthetized animals is not a wholly reliable test.

In dog 96 a gastric fistula was made a month before cutting the second vagus. When this animal was etherized at the time of cutting the second nerve, comparative graphic tracings were made of the motility changes in the stomach after stimulating the normal and the regenerating nerve. Under light anesthesia, stimulation of the normal vagus caused a strong contraction followed by smaller peristaltic waves. Stimulation of the regenerating vagus a few minutes later caused no detectable contraction of the stomach (fig. 1). Two weeks after cutting the second vagus, tracings were made of the hunger contractions in this dog (fig. 2). These were similar to those occurring before cutting the second nerve but not so vigorous. These contrac-

TABLE 3

Dog 95. Adult brown bull dog

March 7, 1918. Sectioned the right vago-sympathetic and dropped the ends together in the carotid sheath. The sheath was then closed by two stitches, but none were taken through the nerve ends

November 5, 1919. The left vago-sympathetic was sectioned

DATE	HOUR	PROCEEDING	RESPIRATION	PULSE	REMARKS
Nov. 5	1:00 p.m.	Before operating	15	74	Dog active
Nov. 5	2:00 p.m.	Cut left vago-sympathetic			
Nov. 6			14	147	Eating. Chases guinea pig
Nov. 7			12	142	Drinks water; vomits solid food
Nov. 15			12	124	Eating and vomiting
Nov. 17		Dog emaciated	15	146	Drinks milk
Nov. 18			12	112	Frequent hiccough
Nov. 19	2:00 p.m.		12	108	Dog shivering, hiccough
Nov. 19	2:45 p.m.	Given 0.3 cc. of atropine sulph.			
Nov. 19	3:15 p.m.		12	186	
Nov. 19	7:30 p.m.		12	120	
Nov. 20			16	129	Dog eats and then vomits. Is very thin
Nov. 21		Dog is fed soft food only			Vomiting reduced
Dec. 1					Continued progressive emaciation
Dec. 8		Will not eat			Lies quietly; does not move about
Dec. 9		Thorax opened, vagi stimulated			Dog comatose
Dec. 9		Autopsy			

Lungs have scattered hyperemic areas but no consolidation. One small pus pocket found. Stomach empty, normal appearance. Heart normal. The two ends of left vagus united by a smaller strand of tissue.

tions were promptly abolished by atropine. This is suggestive of direct paralysis of the vagus fibers in the regenerating nerve but it does not prove that regeneration of the gastro-motor fibers had occurred, for this inhibition might have been due to any of the following possible factors: *a*, a direct action on the intrinsic plexuses as suggested by Magnus for the intestine; *b*, inhibition through the splanchnics as result of central stimulation by the atropine; *c*, or some possible relation to the secretion of epinephrin. All other evidence in this dog indicated that these gastric fibers were not functional and hence the observation indicates that atropine will inhibit gastric contractions independently of whether or not the vagus fibers are active. At any rate, electric stimulation of the regenerating nerve in the anesthetized animal gave no gastric motor effects and atropine abolished gastric motility in the unanesthetized condition.

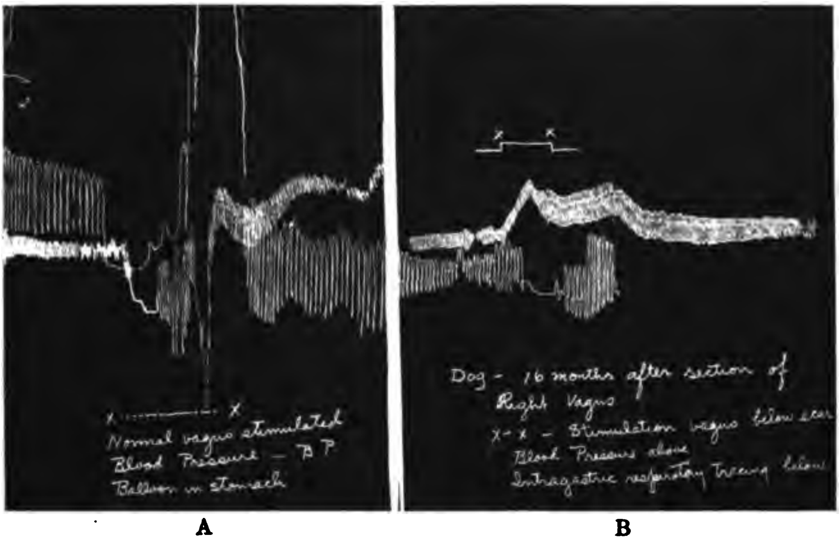

Fig. 1. A. Stimulation of normal vagus, dog 96. Carotid blood pressure and balloon in stomach to record respiration and stomach contractions. The stomach tracing is above the blood pressure record on the left side of figure A and drops below on the right side. Cardiac inhibition, respiratory inhibition and contraction of the stomach followed stimulation of the nerve.

B. Stimulation of the right vagus sixteen months after section and approximation of the ends of the nerve. No cardiac inhibition, no stomach contraction, inhibition of respiration and a rise in blood pressure followed stimulation of the nerve below the scar of union. Ether used as anesthetic.

In these dogs in which a year and a half was allowed for the regeneration of one vagus after section and suture, the subsequent division of the remaining nerve was followed by no appreciable change in the rate of breathing or in the amplitude of the respiratory movements, so far as could be judged by ocular observation. Stimulation above and below the scar of suture with a tetanizing current caused the usual inhibition of breathing. Although causing no cardiac inhibition when electrically stimulated in the etherized dog there was an immediate pressor effect on the blood pressure (fig. 1). Regeneration of afferent fibers in the vagus had therefore occurred.

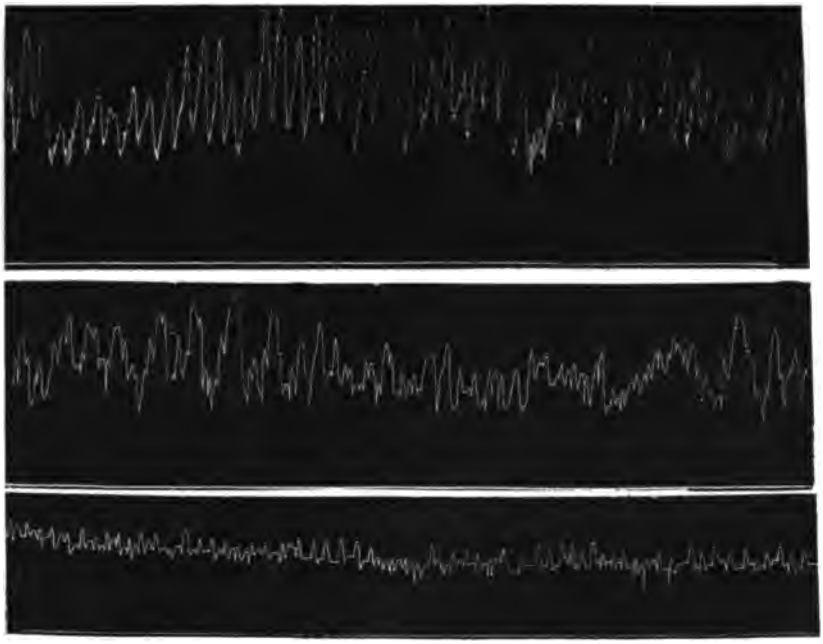

Fig. 2. Tracings numbered I, II, and III in order from above, downwards. *I*. Gastric hunger contractions, dog 96, October 28. Right vagus sectioned and sutured sixteen months previously. Left vagus intact.

II. November 11, gastric hunger contractions after cutting left vagus leaving only the regenerating nerve intact.

III. November 11. Continuation of tracing *II*. Thirty minutes after a subcutaneous injection of 0.3 cc. of 0.1 per cent atropine sulphate.

After death the regenerated nerve of dog 95 was excised for a distance of half an inch above and below the point of suture. This was stained by Ranson's pyridine silver method for medullated and nonmedullated fibers. Save that the arrangement of nerve fibers in fascicles below the scar was not evident there was no distinct difference in the number of nerve fibers in the regenerated part as compared with that above the point of section.

In this report no reference is made to the changes in the sympathetic nerves of the neck which were cut simultaneously with the vagi.

SUMMARY

One vagus nerve was sectioned and the ends approximated so as to allow regeneration to occur in a series of dogs and cats. The regenerating fibers were stimulated electrically at time intervals varying from one to sixteen months after cutting. These tests made with the animals under ether anesthesia gave no evidence of the regeneration of either cardiac inhibitory or gastric motor fibers.

In one dog twenty months after one vagus was sectioned, this nerve was stimulated with the dog in a comatose condition but no ether anesthesia. Distinct cardiac inhibition followed.

In two dogs, section of the remaining normal vagus, sixteen and twenty months after previously sectioning and suturing the other, led to death in sixteen and thirty-four days respectively. Apparently death was due to starvation resulting from difficulty in swallowing and frequent vomiting. During the period of life following section of the second vagus, the following facts were noted:

1. An immediate marked increase in pulse rate followed section of the second vagus. This slowly declined and after eleven to fourteen days the rate was that of a normal animal. At this stage atropine caused a great increase in the rate of the heart beat. These effects occurred in a dog in which the regenerating nerve was not functional for subsequent division of the nerve caused no change in the heart rate.

2. With only the regenerating nerve intact, but with no evidence of it being functional, atropine reduced the gastric motility.

3. The rate of breathing with only the regenerating nerve intact was the same as it was with one vagus intact. Cutting the regenerating nerve led to the classic picture of slow labored breathing. Stimulation of the regenerating nerve above and below the scar caused the normal respiratory inhibition and pressor effects on the blood pressure. Regeneration of the vagus fibers necessary to maintain the normal

respiratory rhythm had therefore occurred. Whether these were motor to the larynx or afferent from the lungs was not determined.

After bilateral vagotomy, some compensatory process is set up whereby the pulse rate is brought back to normal in spite of the absence of the vagi.

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EFFECT OF VARIOUS SUBSTANCES UPON THE COAGULATION OF CITRATED PLASMA[1]

BEN KARPMAN

From the Department of Pharmacology, University of Minnesota

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Although an enormous literature has arisen upon the coagulation of the blood, and the rôle of inorganic salts, lipoids and tissue extracts has been extensively investigated, little has been done along the line of the organic substances. I have therefore undertaken, in this paper, a systematic investigation of the effects of as many classes of organic compounds as possible upon the coagulation of citrated blood plasma. It was hoped to determine by this method whether any substances or radicals could be found which might be regarded as specifically favoring or specifically inhibiting the act of coagulation, and which might thus throw light upon the intimate chemical mechanism of coagulation.

METHOD

Fresh beef blood taken from the abattoir at the time of slaughter was put into a jar containing enough sodium citrate solution to make the final mixture contain 0.5 per cent of the salt. This was centrifuged, put in the ice box and used the same day.[2] A 1 per cent solution of

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[1] Thesis submitted for the degree of M.D., University of Minnesota.

[2] Effect of age on the activity of citrated plasma. I. *Citrated plasma* was tested for coagulation time,—when fresh, and also when 1, 2, and 3 days old. Coagulation time was found to be 5 minutes, 35 seconds; 8 minutes; and 7 minutes, 15 seconds, respectively. The precipitate formed during these intervals was not removed.

II. Citrated plasma was tested for coagulation time when fresh, 11 days, 14 days, 16 days and 21 days old. Precipitate formed removed in the intervals mentioned. Coagulation time was 5 minutes, 35 seconds, and 4 hours for the first two; the rest formed within a day a small amount of solid material which was suspended in the fluid.

III. Citrated plasma was divided into three portions and tested for coagulation time in the intervals indicated. Precipitate removed each time. Normal clotting time, 13 minutes.

a. Tested when 2, 6, 7, 8 and 9 days old. Coagulation time 15 minutes, 55 secdry crystals of $CaCl_2$ in distilled water was made, and this was added to the plasma at the same time as the substance whose effect on coagulation was being studied.

The tubes used were mostly small Wassermann tubes. Where larger tubes were used they were, whenever possible, selected of the same size to insure uniformity of contact with foreign material. Observations were all made at room temperature, usually 72°F. It is to be noted, however, that during the winter and spring months the average coagulation time of controls was 8.1 minutes (the highest being 13.4 minutes and the lowest being 3.6 minutes), while during the summer months the average coagulation time of the plasma was 5.6 minutes

onds; 19 minutes, 15 seconds; 21 minutes, 36 seconds; 3 hours and 6 hours respectively.

b. Tested when 6 and 8 days old. The first gave a coagulation time of 40 minutes, 15 seconds; the second was still fluid after 12 hours.

c. Tested when 8 days old. Still fluid after 24 hours.

IV. Citrated plasma was tested for coagulation time when fresh, 4 days, 5 days, 9 days and 10 days old. At the intervals indicated, the citrated plasma was divided into two portions; a larger one containing the clear supernatant fluid and a smaller one containing all of the precipitate formed during the interval. The former was treated again in the same manner on successive days as more precipitate formed. Normal coagulation time 4 minutes, 10 seconds. The larger (supernatant) fraction gave a coagulation time of 3 minutes, 24 seconds; 8 minutes, 28 seconds, and 8 minutes, 42 seconds, on the 4th, 9th and 10th days respectively. The first small (precipitate-containing) portion gave a coagulation time of 2 minutes, 37 seconds on the 4th day, and spontaneous clotting on the 5th day; the second small portion, 3 minutes, 47 seconds on the 9th day; the third small portion, 5 minutes, 52 seconds on the 10th day.

V. An extract was prepared from fresh citrated plasma by a method modified from Wright (1). The plasma to which this extract was added showed some reduction in coagulation time. The results were vitiated by the fact that the extract was used in a solution of sodium carbonate; as the latter substance interferes with coagulation, it masked the effect of the extract.

The above observations suggest that the coagulation time of citrated plasma increases considerably with age. This increase is more marked when the precipitate formed in the intervals is removed, but when the precipitate is present in larger amounts, the coagulation time is markedly shorter. This fact suggests that the precipitate contains something which aids coagulation; but whether this is due to the presence in the precipitate of a definite thromboplastic substance, or is merely due to hydrolytic dissociation of calcium citrate with liberation of calcium ions, or to some other entirely different factor, has not been determined. A substance extracted from citrated plasma also showed coagulating power; but it is not clear yet whether this substance is identical with other kephalin like substances that can similarly be extracted from various organs and tissues.

(the highest being 8 minutes and lowest being 2.3 minutes). Whether such marked variation is due to heat or to some nutritional factor that in some way affects coagulation time in winter as compared with summer, has not been determined. To insure uniformity and greater accuracy, the plasma, $CaCl_2$ solution and the substance used (when liquid) were all measured from microburettes graduated to 0.1 cc. The plasma was measured out first and then the Ca solution was added. Immediately afterward the chemical to be tested was added, and the tube moderately shaken and mixed. The end point was judged by Howell's method inverting the tube, and time noted in minutes and seconds. Occasionally we experienced a little difficulty with this criterion, since some of the substances would give only partial or incomplete coagulation.

A new control tube was used for each set of experiments and for each sample of plasma.

Throughout the experiments care was taken to test the effects of single substances rather than of mixtures, but this was not always possible, a fact which must be borne in mind in the interpretation of results. The effect of water on coagulation time of plasma has been determined in a series of experiments, and it was found that there is a definite, although not very marked, diminution in coagulation time. It is clear, however, that these results cannot be applied entirely to solutions of substances, since these in solutions behave somewhat differently than when pure, even aside from the mere factor of water. Where mixed salts were used, such as choline HCl or tyramine HCl, the results cannot be applied entirely to choline or tyramine without reservation, since the acid factor might mask their effect.

In the descriptions which follow, the word "clot" is used to designate the formation of a single coherent jelly-like mass which adheres to the walls of the tube; the word "precipitate" is used to designate the formation of discrete flocculi which do not cohere to one another and do not form a jelly. This use of these terms must be borne in mind in the interpretation of the results recorded in this paper.

RESULTS OF EXPERIMENTS

Group I. Aliphatic series

1. Formic acid. All tubes formed thick viscous fluids. No distinct precipitate could be seen. The time of solidification (coagulation) was in direct proportion to concentration. The solid resembled more a precipitate than a clot and appeared homogeneous and structureless.

2. Acetic acid. All tubes gave a very flocculent precipitate, the amount of the latter being in direct proportion to concentration. With the exception of the 0.8 per cent which remained permanently fluid, the supernatant fluid of the rest of the tubes solidified after a few days; it was of a soft mushy consistency and no fluid could be expressed from it.

3. Propionic acid. All tubes became cloudy with the formation of a precipitate. The 0.85 per cent tube was still fluid after 2 hours; the rest of the tubes solidified in 2 minutes, 10 seconds; 15 seconds and 5 seconds, (control 5 minutes, 45 seconds), the substance being very soft and mushy.

4. Butyric acid. The 0.85 per cent became cloudy and emulsion-like with a fine flocculent precipitate, and remained permanently fluid. The 2 per cent gave a very soft solid after 2 days; the rest of the tubes formed almost instantaneously thick solid precipitates.

5. Valeric acid. The 0.4 per cent mixed well but remained permanently fluid with formation of a precipitate and cloudy supernatant fluid. The rest of the tubes did not mix well, forming thick emulsion-like fluids above and a large precipitate on the bottom. Of these, the 0.85 per cent was still fluid after 3 days; the 2 per cent was practically solid in 15 minutes; and the 4 per cent and 7.5 per cent formed almost instantaneously thick solid precipitates.

6. Oleic acid. On mixing an emulsion formed in the tubes. The contents of all tubes were still fluid after 24 hours with an evident tendency to form solid emulsions.

7. Formaldehyde. The clots were very soft, practically semi-solid[8] and very transparent, the transparency being more marked in higher concentrations. No precipitate was visible and no fluid could be expressed. The solid was easily broken up into small particles.

8. Alcohol. All tubes became cloudy and milky in proportion to concentration, and a white precipitate separated out afterwards. The 7.5 per cent and 14 per cent tubes solidified in time equal to that of control. The other tubes remained permanently fluid. In firmness the clots resembled the normal.

9. Glycerine. In concentration of 0.85 per cent to 3 per cent showed some retardation, this being more marked in the lower percentages; in concentration between 4 per cent and 7.5 per cent no marked deviation from control was evident, while in concentration above 7.5 per cent it again showed retardation, the effect increasing with increased concentration. The clots were very firm and elastic, and no serum could be expressed.[4]

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[8] See footnote 4.

10. Ether. The 7.5 per cent and the 14 per cent mixtures showed considerable retardation, the clots resembling normal in firmness and consistency. The 20 per cent and 30 per cent coagulated but partly in 30 minutes, the rest remaining fluid. As the substance was added it did not mix with the plasma and was seen gradually to rise to the top. In all cases, coagulation proceeded from the bottom.

11. Acetone. The 4 per cent, 7.5 per cent and 14 per cent tubes formed clear mixtures, which coagulated in 1 hour, 1.8 hour and 2.5 hours respectively. The 20 per cent, 25 per cent and 29 per cent formed turbid, emulsion-like mixtures with precipitation. The first two were found to be solid after 2 days, while the last one remained fluid. The clots were soft and mushy and no fluid could be expressed.

12. Urea. There was no precipitate visible. Clot softer than normal. Considerable retardation.

13. Hexamethylenamine. In all tubes, with the exception of 0.1 per cent, there was a settlement of the substance in proportion to concentration. There was no apparent difference whether it was used in solution or in pure form, for although the deviations in the latter were much more marked, coagulation time was practically normal. Clots were somewhat harder.

14. Hexamethylenamine and phosphoric acid, 50 per cent of each. All tubes were fluid at first with no evident signs of either coagulation or precipitation. In 2½ hours became thick and semi-solid. Were all found to be solid in 15 hours, soft in consistency, with no fluid expressible, the solid resembling more a precipitate than a clot.

15. Choline hydrochloride. All tubes gave delayed coagulation time in proportion to concentration. The 5 per cent was still fluid after a half-hour.

16. Glycocol. In small concentrations up to 3 per cent the tubes became cloudy while in higher concentrations the tubes formed a distinct precipitate. After 18 hours the 0.3 per cent was solid, the 0.6 per cent semi-solid, the rest solid white precipitates.

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[4] In the course of the experiments it was frequently noted that there was a considerable difference in the effect of various substances on the character of the clot. Thus, glycerine gave a very firm and elastic clot; dinitrobenzol a soft clot, while resorcin gave a firm clot in concentration up to 0.5 per cent and a very soft one in higher percentages, and no fluid could be expressed.

17. Chloroform. Did not mix with the plasma and was seen floating in it as oily drops, gradually settling to the bottom. Coagulation proceeded from the top. The clots were soft, clear and jelly-like.

1. The first members of the saturated fatty acid series show considerable similarity in their effect on coagulation of plasma. They all interfered with coagulation by precipitation, the amount of the latter being in direct proportion to concentration; and the higher the member the more pronounced was the reaction.

2. Oleic acid interfered with coagulation by emulsification.

3. The representative members of the other group, namely, formaldehyde, alcohol, ether and acetone, have all produced the effect of retardation, interference, or both. The retardation produced by formaldehyde can hardly be explained by precipitation since it did not show any visible change; however, the character and consistency of the solid suggest that it was more like a precipitate than a clot.

4. Glycerine did not show any appreciable effect on coagulation, although its power of retarding coagulation in very low and very high concentration is suggestive.

Of the rest, glycocol markedly interfered with coagulation even in small concentrations. The effect of choline HCl and urea is considerable less marked while hexamethylenamine was practically without any effect. Chloroform has shown a definite retardation and possible inhibition.

Group II. Aromatic Series

1. Benzol. On mixing, all tubes assumed a milky emulsion-like appearance. The tubes solidified later, some showing a separation into three layers, benzol, emulsion and plasma, from the top down.

2. Phenol. In concentrations of 0.2 per cent to 0.85 per cent, normal or only partial coagulation was obtained, whether the phenol was used in pure form or in solution, although the process was more complete when the same strength was used in solution than in crystals. The clot formed was normal in appearance and spread from the top down. The 2 per cent to 8.5 per cent showed emulsification and precipitation, solidifying later with separation of fluid.

3. Resorcin. The 0.1 per cent to 0.4 per cent gave firm clots with delayed coagulation time; the 0.85 per cent gave a soft clot. The 2 per cent to 25 per cent assumed an emulsion-like appearance with subsequent separation of a precipitate, not unlike phenol, but the tubes remaining permanently fluid.

4. Benzaldehyde. On mixing all tubes became turbid and emulsion-like with formation of a precipitate, the thickness of the emulsion and the amount of the precipitate being in direct proportion to concentration. All tubes remained permanently fluid except the 0.85 per cent, which solidified very slowly.

5. Benzoic acid. The 0.1 per cent to 0.4 per cent and 2 per cent showed delayed or partial coagulation, in direct proportion to concentration, the clots being less elastic. The 0.85 per cent gave a precipitate and remained fluid.

6. Benzyl alcohol. All tubes showed emulsification and precipitation, in direct proportion to concentration.

7. Nitrobenzol. On mixing, all tubes became turbid, separating later a precipitate which settled to the bottom as a white solid mass, the amount of the precipitate being in direct proportion to concentration. The supernatant fluid was quite clear and coagulated, the coagulation time not varying markedly from normal.

8. Dinitrobenzol. In spite of shaking, a considerable part of the crystals settled to the bottom. Coagulation time was normal. No precipitate was visible.

9. Cinnamic acid. The 0.85 per cent gave normal coagulation time. The 2 per cent to 4 per cent formed a hard surface scum, the material below remaining fluid for a considerable time. Were all found to be clotted in 60 hours, the clots being paler in appearance and not as firm in consistency as normal.

10. Aniline. The 0.85 per cent gave delayed coagulation, the 2 per cent partial coagulation. The 2.4 per cent to 29 per cent formed emulsion-like mixtures.

11. Phenylhydrazine. On the addition of the substance there was an almost instantaneous precipitation and solidification, the solid being soft and mushy with no fluid expressible. The solid was very much unlike a clot and was easily broken up into small masses.

12. Pyridine. Tubes became cloudy, pale and soon solidified, the solid being easily broken up into small bits and masses, and resembling more a precipitate. No fluid could be expressed.

13. Quinoline. All tubes showed a precipitate on the addition of quinoline, the amount being in direct proportion to the amount of substance used.

14. Tyramine hydrochloride. Clots were apparently normal both as to color and consistency.

15. Antipyrine. All tubes formed a clear solution with no settlement of antipyrine or precipitate visible. The 4 per cent was still fluid after a week, the 7.5 per cent was solid in 14 hours, the solid being very soft and flocculent. The rest solidified promptly on the addition of the substance, the solids being of soft gelatinous consistency and with no fluid expressible. The retardation was inversely proportional to concentration.

16. Caffeine. There was a settlement of caffeine on the bottom in proportion to concentration. Clots very clear, soft and no fluid could be expressed.

The benzol series showed a variable effect:

1. Benzol, aniline and benzaldehyde interfered with coagulation by emulsification. With phenol the effect is changed to that of precipitation, due to a change in solubility by the introduction of the OH group. This effect is considerably weakened by the introduction of $(OH)_2$, since resorcin even in very high concentrations did not give sufficient precipitation to cause solidification, an effect which phenol produced in much lower concentration. This difference is also shown by the fact that in very low concentrations resorcin showed retardation against incomplete coagulation of phenol for the same concentration. It should also be noted that phenol when used in solution is more effective than when used in pure form.

2. Comparing benzoic and cinnamic acids it is seen that both retard coagulation, but the action of the latter is far more marked than that of the former. Here too, probably, one of the causes of the difference may lie in their different solubilities, cinnamic acid being the less soluble.

3. However, that solubility alone cannot account for all the effect of a substance on coagulation is seen in the case of benzyl alcohol which, although much more soluble than either of the above acids, has shown interference even in small concentrations.

4. Nitrobenzol and dinitrobenzol have shown rather indifferent effect.

5. Phenylhydrazine gave an almost instantaneous solidification in whatever concentration used. The effect of pyridine was somewhat less marked, requiring higher concentrations and more time for solidification; while the quinoline precipitate remained fluid in high concentrations. In the case of these three substances, the difference in action can hardly be explained by differences in solubilities since pyridine is very soluble, quinoline somewhat less, while phenylhydrazine is least of all.

Antipyrine and caffeine have both definitely retarded coagulation, although neither produced any visible change in plasma. Tyramine was practically without any effect.

Group III. Alkaloids

1. Quinine alkaloid, pure. On mixing, all of the quinine came to the top. Clots were all normal in consistency, nor was there any difference in coagulation time as compared with control.

2. Quinine bisulphate. On the addition of the salt, there was an almost instantaneous precipitation and solidification. The 2 per cent precipitate soon settled to the bottom, leaving a fluid above; the 4 per cent formed a solid precipitate throughout.

3. Strychnine alkaloid, pure. On mixing, the tubes became cloudy; later there was a settlement at the bottom in proportion to concentration. In time and consistency the clots were practically normal.

4. Atropin alkaloid, pure. The 0.2 per cent formed a solid scum which on inverting would prevent flowing out, though the rest of the tube remained fluid for a considerable time. Noted to be completely solid after a week. Clot normal, somewhat softer. The 0.4 per cent showed considerable retardation.

5. Nicotine. The 2.4 per cent was still fluid after 17 hours and but partly solidified in a week. The rest of the tubes showed marked retardation in coagulation, which was inversely proportional to concentration. The clots were very soft, mushy, with no body, and but little fluid could be expressed. No precipitate was visible.

Of the alkaloids used quinine and strychnine showed themselves to be without any effect on coagulation of plasma; atropin showed some retardation while nicotine gave most definite and marked retardation. Here the difference in action lies perhaps both in the different solubilities and alkalinities since quinine and strychnine show least of these properties; atropine somewhat more and nicotine most of all.

Quinine bisulphate must, of course, be interpreted in terms of its acid content which interfere with coagulation by precipitation.

Group IV. Inorganic substances

1. Ammonia. On the addition of ammonia, all tubes showed a hazy cloudiness, which within an hour settled to the bottom as a very light precipitate,[5] the amount of the latter was in direct proportion to concentration. The supernatant liquid remained permanently fluid.

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[5] This precipitate was tested and was shown to have the following properties: It was insoluble in water and alkalies and in 80 per cent alcohol; soluble in acids and may be reprecipitated by alkalies; not coagulated by heat in acid solutions. Hence the substance is very likely a metaprotein compound of ammonia.

2. Sodium carbonate. All tubes gave a whitish precipitate. After 4 hours all were still fluid and somewhat gelatinous in appearance. The 0.1 per cent was found to have become solid in 18 hours; the 0.25 per cent and 0.35 per cent in 5 days, the rest remaining fluid.

3. Hydrochloric acid. On the addition of the acid all tubes became immediately cloudy with the formation of a precipitate, the amount of the latter being in proportion to concentration. The 2 per cent and 2.3 per cent were practically all precipitate. After 18 hours the 0.2 per cent and 0.4 per cent were still fluid; the rest of the tubes apparently solid, but the solidity easily disturbed by moderate shaking.

4. Sulphuric acid. On the addition of the acid all tubes became cloudy, some separating later a white precipitate. The 0.2 per cent and 0.3 per cent were found solid after 3 days and after 45 minutes respectively; the rest, 0.4 per cent to 1.25 per cent formed thick viscous fluids which later solidified, the solid being very soft and resembling more a precipitate.

5. Phosphoric acid. All tubes were still fluid after 15 hours; no precipitate visible. They became solid after 10 days, the clot being soft, with no fluid expressible.

In this group, ammonia and HCl have shown marked interference even in small concentrations; Na_2CO_3 and H_2SO_4 showed both retardation and interference, while H_3PO_4 showed the least effect, producing retardation even in high concentrations.

If we now sum up the effect of various chemicals on coagulation of citrated plasma, we may offer provisionally the following grouping.

I. No effect. 1. Some substances such as alkaloids, dinitrobenzol, tyramine hydrochloride, etc., have no effect on coagulation in whatever concentrations used. They do not produce any visible change in the plasma, although some may produce such change (nitrobenzol).

2. Some substances may have no effect in certain concentrations, while producing a definite effect in other concentrations (retardation, etc.) as glycerine, alcohol, etc.

II. Retardation. The effect of retardation may show itself either in prolongation of coagulation time or incompleteness of the process.

1. Prolongation of coagulation time varies considerably with each substance and concentration. In most instances, the degree of retardation was directly proportional to concentration used. In some cases, however, notably with antipyrine and nicotine, this was inversely proportional to concentration; the proportional decrease of retardation with increased concentration may be so progressive that the coagu-

lation time will finally fall below that of the control and thus assume the form of hastening. In yet another case (formic acid) retardation started in lower concentrations in direct proportion to concentration, ending with apparent hastening in higher concentration.

Although some substances do not show any other effect but that of retardation (formaldehyde, etc.), other substances will retard in some concentration while producing a different effect in other concentration (glycerine, resorcin, Na_2CO_3, etc.), most frequently incompleteness or interference with coagulation.

2. Incomplete coagulation. This is not as frequent as the preceding but there are several chemicals which sometimes effect only partial coagulation. As a rule it is accompanied by other effects such as retardation (ether, chloroform, urea, etc.) or precipitation (phenol), and a visible change. From the fact that some cases of incomplete coagulation finally coagulate after a lapse of considerable time, we may regard incomplete coagulation as the next step of a markedly retarded coagulation.

III. Interference. Interference with coagulation may manifest itself in several ways.

1. Precipitation. This is the most common occurrence, the amount of precipitation varying widely with different substances and concentrations used. Thus the members of the saturated fatty acid series, as well as phenylhydrazine, pyridine, HCl, Na_2CO_3, NH_3, benzoic acid, etc., will interfere with coagulation even in very small concentrations; other substances (resorcin, etc.) may require somewhat higher concentrations, while still others (acetone, urea, etc.) require relatively high concentrations to produce the same effect.

2. Emulsification. This is of somewhat less frequent occurrence than the preceding and is noted in such substances as oleic acid, benzol, aniline; others (benzaldehyde, acetone, etc.) show a mixed effect. Both precipitation and emulsification are frequently accompanied by retardation or incomplete coagulation.

3. Inhibition. Such substances as nicotine, antipyrine, caffeine, etc., occasionally show apparent inhibitory effects, i.e., no coagulation takes place and no change in the plasma is visible. It usually accompanies retardation and as a factor it probably stands between retardation and interference. To this group probably belong also chloroform and ether.

IV. Acceleration. We have not encountered a single case of genuine hastening of coagulation. Some substances do hasten the process

TABLE 1

Showing the effect of chemicals on coagulation

	NO EFFECT		RETARDATION				INTERFERENCE			ACCELERATION	
	Per cent used		Per cent used				Per cent used			Per cent used	
		Change in plasma	Prolongation	Incompleteness.	Degree of retardation	Change in plasma	Inhibition	Precipitation	Emulsion		Change in plasma
Group I. Aliphatic series											
Formic acid..........			0.82–3.8		4.0–0.2					7.3–12.5	
Acetic acid...........								0.8–3.9		7.3–12.5	Ppt?
Propionic acid.......								0.83–4.0		7.7–17.2	Ppt?
Butyric acid.........								0.83–7.5			
Valeric acid..........								0.4–7.7			
Oleic acid............									7.7–25.0		
Formaldehyde........			3.1–10.0		2.180						
Alcohol..............	7.7–14.3	Cloudy						20.0–25.0			
			0.85–3.0								
Glycerine............	4.0–7.5		7.5–29.0								
Ether................			7.5–14.3	20.0–29.0	1.8–2.7	Transparent					
Acetone..............			4.0–14.3		6.3–9.4	Turbid		20.0–29.0			
Urea.................			7.5	12.5	3.3					0.8–16.0?	
Hexamethylamine....	0.08–20.0	Settlement									
Hexamethylamine phosphoric acid....			2.0–14.7		200.0						
Choline HCl.........			0.77–3.0	5.0	1.5–4.2		5.0				
Glycocol.............			0.3	0.6	220.0	Cloudy		1.0–10.0			
Chloroform..........			7.7–14.3	20.0–25.0	1.7–8.0						

Group II. Aromatic series

Benzol									13.3–44.0		
Phenol	0.4	Cloudy		0.2–0.8				2.0–8.5			
Resorcin			0.1–1.0		1.7–1.3			2.0–25.0			
Benzaldehyde			0.83		3.0	Turbid			1.6–29.0		
Benzoic acid			0.1–1.6		1.7–8.0	Ppt.					
Benzyl alcohol								0.83–7.7			
Nitrobenzol	7.7–25.0	Ppt.									
Dinitrobenzol	0.83–2.4										
Cinnamic acid	0.83		1.6–4.0		1.0–330.0						
Aniline			0.83		1.4				1.6–29.0		
Phenylhydrazine								7.7–25.0			
Pyridine								4.0–29.0			
Quinoline								2.0–29.0			
Tyramine HCl	0.08–0.5										
Antipyrine			7.7–14.3		130–0.12		4.0			20.0	
Caffeine			0.8–14.3		1.5–150.0					7.7	

Group III. Alkaloids

Quinine	0.4–4.0										
Strychnine	0.2–1.6										
Quinine bisulphate								2.0–4.0			
Atropin			0.2–0.4		1680–1.5						
Nicotine			7.7–29.4	2.4	12.0–1.3						

Group IV. Inorganic chemicals

Ammonia								1.2 –7.5			
Sodium carbonate			0.14–0.33		206–1030	Ppt.		0.4 –0.45			
HCl								0.21–2.3			
H_2SO_4			0.2–0.3					0.4 –1.25			
Phosphoric acid			2.0–14.7		480	Ppt.					

more than the controls (phenylhydrazine, pyridine, etc.) but the character of the solid formed resembles more a precipitate than a clot, although no distinct precipitate is visible. It is possible that, as in case of interference, there is more than one mechanism by which coagulation is brought about.

It should be said here that some substances belong properly to this group although not causing any visible change (formic acid, formaldehyde, etc.). There are good reasons to believe that although no change is visible, their apparent retardation is really an interference.

It seems thus quite clear from the above considerations that a close relation exists between the various factors discussed. Thus, substances having no effect on coagulation of plasma in certain concentrations will, in other concentrations, retard the process; while substances retarding in some concentrations will, in higher concentrations, interfere or inhibit. These phases are apparently intimately related, one often passing insensibly into the other, forming a progressive chain of events (no effect—retardation—incomplete coagulation—inhibition—interference) and all probably operating on the basis of some common property.

What then are the conditions that will determine the particular effect of a substance upon coagulation of plasma?

The substances having no appreciable effect on coagulation may act in a particular manner because of one or more of the following reasons.

1. They are not soluble in plasma (alkaloids, dinitrobenzol).
2. They may be soluble in water, but the medium is not favorable for their action (formin requires an acid medium).
3. Their effect is neutralized by an opposite property (tyramine HCl-acid-alkaline-neutral.)
4. The changes produced do not sufficiently alter the plasma so as to interfere with coagulation.

As we pass to the next phase, that of retardation, it would seem that the retarding substance induces certain definite changes in the plasma and these may be due to any one or more of the following conditions:

1. The substances are not soluble in plasma.
2. They do not react chemically with plasma nor form easily a physical mixture (chloroform, ether).
3. They act by dehydration, absorbing water from plasma (glycerine).
4. Their acid or alkaline reaction (caffeine, antipyrine, nicotine, urea, Na_2CO_3).

5. Their effects neutralized by an opposite property, cholin HCl.
6. May be strong reducing agent (formaldehyde).

The same mechanism is probably at work in case of interference, only the changes are so pronounced as to interfere entirely with the process of coagulation:

1. Many of the substances are quite soluble and, uniting with some substances of the plasma, cause precipitation (phenol, resorcin).
2. Their reaction is markedly acid or alkaline (the fatty acids, HCl, benzoic acid, pyridine, ammonia, Na_2CO_3, etc.). Here evidently solubility in water as such does not play an important rôle, for the effect is the same whether the substances are very soluble in water—as pyridine, HCl, etc.—or increasingly less soluble—as the fatty acids.
3. They may be insoluble in water but form an emulsion, thus depriving the plasma of water (oleic acid, benzol, etc.).
4. They may have a marked solvent or precipitant action on some plasma constituent (alcohol, acetone).
5. Being neither soluble in plasma nor active chemically, their mere presence inhibits coagulation by preventing the aggregation of fibrin threads and crystals into gel formation (chloroform, ether).

That the precipitate is most likely a new chemical compound is readily seen from a qualitative analysis of one of the precipitates (ammonia q.v.); a further analysis of each individual precipitate formed would probably show that the precipitate formed in each case is different. Protein behaves in an acid solution like a cation, and anions render it insoluble; in an alkaline medium, it behaves like an anion, migrating to the anode, and cations render it insoluble. Although in general the rate of precipitation is proportional, *ceteris paribus*, to the molecular conductivity of the added salt, it would seem that while it is true for the inorganic acids, it is not exactly true for the fatty acids, for their ionization decreases as we go up the scale while the precipitation increases at the same time (2).

The process of emulsification is a much simpler one and the disturbance is more of a physical than of a chemical nature.

As it has been observed in numerous instances that the reaction between the substance used and the plasma is a quantitative one, the degree of reaction will obviously depend partly on the properties of the substance, and on the condition of the plasma. It is quite evident that the more soluble a substance is, the less likely it is, *ceteris paribus*, to interfere with coagulation. Thus, the precipitating action of the fatty acids rises as we ascend the scale, while their solubility decreases at the same time. Phenol in solution interferes with coagulation much less than when used in crystal form, while resorcin interferes less than phenol. Quinine and strychnine are insoluble and have no effect. Finally, it may be said that in general the substances belonging to the second group are considerably more soluble than those of the third. This, perhaps, will explain why a substance will interfere in higher concentrations while only retarding in lower concentrations, since smaller quantities are more easily dissolved. On the other hand, that water per se is not the determining factor is quite evident from the consideration that some substances interfere markedly with coagulation, although they are very soluble (phenylhydrazine, urea, pyridine, etc.).

It has long been observed that the reaction of the blood has a considerable effect on its coagulability. An increased acidity leads to an increased aggregation and finally precipitation of colloidal particles of fibrin, and similarly increased alkalinity may in smaller concentration change the form of the clot from a crystalline form to a structureless mass, and in higher concentrations cause a total failure of clotting (3).

The present work abundantly verifies these observations. Substances having a distinctly acid or alkaline reaction have, in all cases, failed to cause normal clotting, even when used in smaller concentrations. If the alkaloids are cited as exceptions, it should be remembered that those that had no effect on clotting are totally insoluble (quinine and strychnine) while the more soluble ones had a distinct effect (atropine and nicotine). It is possible that the manner in which acids or alkalies interfere with coagulation is in a way comparable to coagulation of protein by heat, since coagulation of blood is due to the formation of an insoluble fibrin compound. According to Chick and Martin (4), in heat coagulation of protein there is first a denaturation or reaction between the protein and hot water, and second, agglutination or separation of the altered protein in a particulate form, the reaction velocity increasing with an increase in acidity or alkalinity. From the purely physical point of view the addition of acids or alkaline ought to disturb the plasma equilibrium since protein salts have a greater attraction for water than electrically neutral protein and, according to Fisher (5), the presence of acid or alkali greatly increases the power of protein to imbibe water. The semi-solid character of some of the clots and the inability to express water from them is probably due to absorption of water by fibrinogen and hence may be regarded as an incomplete clot.

It is also a common observation in the laboratory that old poorly-preserved kephalin loses its thromboplastic properties and may even retard coagulation; and according to McLean (6) the loss of thromboplastic power goes hand in hand with the development of acid reaction. According to W. H. Heard (7) certain concentrations of alkaline earth cause marked retardations of coagulation. It is conceivable that these variations may be accounted for by variations in their respective hydroxyl ions.

Whether certain chemical groupings have a more intimate relation to coagulation of plasma than others, cannot be said definitely. The introduction of either H or OH ions, as stated above, definitely interferes with coagulation; the introduction of the phenolic OH seemingly has a favorable effect on coagulation; while the presence of nitrogen group by effecting a change in the reaction of the substance, interferes with the process.

SUMMARY

The effect of various chemicals on coagulation of citrated plasma has been studied and it has been shown that a chemical may have one of the following effects on plasma.

1. No effect.
2. Retardation of the process by:
a. Prolongation of the coagulation time.
b. Incomplete or partial coagulation.
3. Interference by:
a. Inhibition.
b. Precipitation.
c. Emulsification.
4. Acceleration.

Reasons have been advanced to show that an intimate relation exists between the factors mentioned and by gradations one may pass into another, suggesting that they all probably work by reason of some common mechanism.

The various properties which may be responsible for the particular effect a substance will have on coagulation have been considered and it was suggested that such effect may depend on the solubility of the substance in plasma, its alkaline or acid reaction, on its dehydrating power, reducing power, etc.

It has also been observed that in the interaction between the chemical and the plasma, new chemical compounds are formed, and the relation is probably a quantitative one.

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THE INFLUENCE OF PITUITARY EXTRACTS ON THE ABSORPTION OF WATER FROM THE SMALL INTESTINE

MAURICE H. REES

From the Physiological Laboratory of University of South Dakota

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Recent investigations in both the experimental and in the clinical fields indicate quite conclusively that pituitary extracts produce at least a temporary (seven to eight hours) antidiuretic effect when administered subcutaneously. The question arises as to how this antidiuretic action is brought about. Is it a direct or an indirect action on kidney excretion?

Motzfeldt (1) concludes from his experiments on rabbits that pituitary extracts produce an antidiuretic action by stimulating the sympathetic nervous system and bringing about a vasoconstriction within the kidney.

Dale (2) working with perfused kidneys of the dog and the cat, found that pituitary extracts caused a vasoconstriction of the renal vessels. Houghton and Merrill (3) arrived at a similar conclusion. On the other hand, King and Stoland (4) found a vasodilatation of the renal vessels and an increased flow of urine.

The literature regarding the effect of pituitary extracts on the intestine is rather contradictory. Fodera and Pittau (5) in 1909 noted that intravenous injections caused defecation. Increased peristaltic waves following intravenous injections were noted by Bell (6) and by Ott and Scott (7). Shamoff (8), working on isolated loops of the rabbit's intestine, found that posterior lobe extracts gave a relaxation of the intestine.

The writer suggested in previous work (9) that the antidiuretic action of pituitary extracts may be due to an interference with the absorption of water from the intestine. It was noted that rabbits quickly developed a diarrhea following subcutaneous injection of pituitary extracts. Cats showed a marked tendency to vomit following similar injections. These observations suggested the advisability of investigating the effect of pituitary extracts on the absorption rate of the intestine and also on the emptying time of the stomach.

In the present work we have attempted to find out whether subcutaneous injections of pituitary extracts cause any variation in the rate of water absorption from the small bowel.

METHODS AND RESULTS

Dogs and cats were used in our experiments. One commercial pituitary extract was used, namely, pituitrin (Parke, Davis & Company).

The injections of pituitrin were subcutaneous in every case, and were given four to five minutes before the beginning of the test experiments, that is, at the close of the control period.

In the experiments recorded in tables 1, 2 and 4 the animals were kept under an anesthetic (ether) during the entire experiment.

The small bowel was exposed with as little trauma as possible, and the lumen was washed with warm tap water. A measured amount of warm tap water was then introduced into the cannulated loop of the bowel. At the end of a half-hour period the water remaining in the bowel was removed and measured, and amount of absorption noted. Four or five minutes before the close of this control period the test animals received a subcutaneous injection of pituitrin. The same amount of warm tap water was introduced into the loop of bowel at the beginning of the second and at the beginning of the third half-hour periods and the amount of absorption noted in each case.

In table 1 (first period) it will be noted that normal rate of absorption varies widely in different animals. This is probably due in part to the varied lengths of bowel used.

Following the injections of pituitrin there was delayed absorption in all but two of the fourteen dogs experimented upon. In one of these two dogs (no. 10) the amount of pituitrin used was probably too small to be effective. In the other case (no. 8) the intestinal mucosa was found to be greatly inflamed and this may account for the failure of the pituitrin to delay absorption.

With cats the results were not so uniform since only four out of the six experimented upon showed a delayed absorption after pituitrin injections. The intestines of cats are much more susceptible to trauma than are those of dogs. This may have been a factor in the variation.

The question arises as to whether the decreased absorption noted in the second and third half-hour periods may not be due to the continua-

TABLE 1

Summary of experiments on the effect of pituitary extracts on the rate of absorption of water from the small intestine. The second column of the table shows the amount of water injected into the washed bowel at the beginning of each 30-minute period. Dogs were used except in nos. 15 to 20 in which cats were used. The pituitrin was injected subcutaneously at the close of each control period

NUMBER OF EXPERIMENT	AMOUNT OF WATER INJECTED	AMOUNT OF PITUITRIN INJECTED	FIRST PERIOD CONTROL		SECOND PERIOD		THIRD PERIOD	
			Water absorbed		Water absorbed		Water absorbed	
			First loop	Second loop	First loop	Second loop	First loop	Second loop
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
1	250	1.0	200.0	210	160.0	175	155	170
2	250	1.0	120.0	155	115.0	135	100	102
3	300	0.5	255.0	255	235.0	240		
4	200	0.5	100.0	90	70.0	68		
5	100	0.5	60.0	58	50.0	55		
6	200	1.0	125.0		100.0		86	
7	300	1.0	192.0		170.0		120	
8	150	0.5	100.0		105.0			
9	200	0.5	40.0		30.0			
10	100	0.25	62.0		75.0			
11	100	0.5	85.0		18.0			
12	200	1.0	130.0		105.0		100	
13	300	1.0	160.0		135.0		120	
14	300	1.0	170.0		130.0		100	
15	25	0.5	21.5		13.5		12	
16	25	0.5	21.0		14.0		11	
17	50	1.0	31.0		31.5		27	
18	50	1.0	33.5		41		26	
19	50	1.0	12.0		0		7	
20	50	1.0	15.0		6.0		5	

tion of the anesthetic and to the operative procedure. To determine this point we took the normal absorption rate on several animals without pituitrin injections. An inspection of table 2 will show that in the absence of any pituitrin injections the absorption rate may be even greater in the second and third half-hour periods than it is in the first half-hour or control period. In no case was there a marked decrease in the second period, and in only one case (no. 21) was there a marked decrease in the third period.

In order that we might still further rule out the possible effect of the anesthetic we repeated the absorption experiments on four decere-

TABLE 2

Control experiments: To show the normal rate of the absorption of water from the small intestine during the first, second and third half-hour periods of the experiment. Dogs were used in experiments 21, 22 and 23. Cats were used in the remaining experiments

NUMBER OF EXPERIMENT	AMOUNT OF WATER INJECTED	AMOUNT OF WATER ABSORBED PER HALF HOUR		
		First period	Second period	Third period
	cc.	*cc.*	*cc.*	*cc.*
21	200	85.0	88	65.0
22	50	42.0	43	32.0
23	50	42.0	40	28.0
24	25	13.0	15	13.5
25	25	8.0	7	14.0
26	25	12.5	15	10
27	25	9.0	8	10.0

TABLE 3

Showing the rate of water absorption from the small intestine before and after the injection of pituitrin in decerebrated dogs. The dogs were decerebrated three hours previous to the beginning of the experiments on absorption.

NUMBER OF EXPERIMENT	AMOUNT OF WATER INTRODUCED	AMOUNT OF PITUITRIN INJECTED	AMOUNT OF WATER ABSORBED PER HALF HOUR		
			First half-hour period, control	Second half-hour period	Third half-hour period
	cc.	*cc.*	*cc.*	*cc.*	*cc.*
28	300	0.50	150	135	100
29	300	0.50	135	100	85
30	450	1.00	225	60	
31	200	0.50	125	20	14

TABLE 4

Effect of pituitary extract on the flow of blood from the mesenteric veins; 0.5 cc. of pituitrin was injected subcutaneously in each experiment. Dogs were used

NUMBER OF EXPERIMENT	BEFORE INJECTION OF PITUITRIN, CONTROL	FIVE MINUTES AFTER INJECTION OF PITUITRIN
	gtt. per minute	*gtt. per minute*
32	30	26
33	160	80
34	70	61
35	140	130

brated dogs. The findings in these experiments are recorded in table 3. In operating on these animals hemorrhage was kept down to a minimum and sufficient time (three hours) was allowed for the animal to recover from the anesthetic and, in so far as possible, from the shock of the operation. It will be noted that in every case there was a decrease in the absorption rate during the second and third periods, that is, following the injection of pituitrin. This shows that the anesthetic could not have been responsible for the decreased absorption following the pituitrin injections.

It was thought that vasoconstriction of the vessels of the intestinal wall might be a factor in reducing the absorption after pituitrin injections. This possibility was investigated by placing a cannula in one of the mesenteric veins, noting the rate of blood flow by the drop method. By referring to table 4 it will be noted that there is a reduction in the number of drops per minute after pituitrin injection. The reduction was not pronounced except in one case (no. 33); in fact, they do not go much beyond the limit of error due to the difficulty of preventing the blood from clotting in the cannula.

DISCUSSION AND CONCLUSIONS

Our investigation leads us to the conclusion that subcutaneous injections of pituitrin bring about a delay in the absorption of water from the small intestine.

This delay does not seem to be sufficient, in most cases, to entirely account for the delay in the excretion of water from the kidneys which has been found to result from pituitrin injections.

It is possible that the subcutaneous injection of pituitrin may cause some vasoconstriction of the intestinal vessels. This can not be pronounced or very extensive since it has been repeatedly shown and was verified by ourselves in this and previous work, that subcutaneous injections of pituitary extracts do not cause a variation in the general blood pressure.

Motzfeldt (1) suggested that the antidiuretic action of pituitary extracts is due to splanchnic stimulation, causing a vasoconstriction within the kidneys. It is possible that this mild splanchnic stimulation also extends to the vessels of the intestine.

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THE SPECIFIC INFLUENCE OF THE ACCELERATOR NERVES ON THE DURATION OF VENTRICULAR SYSTOLE

CARL J. WIGGERS AND LOUIS N. KATZ

From the Physiology Laboratory of Western Reserve University School of Medicine, Cleveland

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INTRODUCTION—PREVIOUS WORK

Ever since the discovery of the accelerator nerves in 1867 by v. Bezold and Bever (1) and the Cyon brothers (2), a sporadic interest has been manifested in the question as to whether, in addition to altering the heart rate, these nerves also specifically affect the strength and duration of ventricular contraction. It does not seem to have been realized, however, that a careful study of their effects on the duration of ventricular systole is capable of disclosing whether the normal ventricular beat is controlled entirely in a mechanical way or whether it is also specifically controllable through nervous influences.

The idea that the mammalian ventricle is controlled in a simple mechanical fashion received its greatest support from the oncometer experiments of Henderson and his co-workers (3). They found that, under normal conditions of venous pressure, the volume curves of the ventricles at all heart rates are practically superimposable on portions of a standard curve obtained during a long vagus beat. This led to the formulation of the law of "uniformity of behavior" accord-to which the systolic volume discharged is entirely a function of the heart rate. Later Henderson and Barringer (4) presented work which indicated that this law also holds when the heart rate is increased by excitation of the accelerator nerves.

Although emphasis has not been specifically laid on the fact by Henderson and his co-workers, it is evidently a corollary of the "uniformity of behavior" law that the duration of systole is fixedly related to the cycle length under all conditions which produce a change in the heart rate. A review of other experimental work indicates, however, that when the accelerator nerves are stimulated, systole and diastole vary quite independently. Thus Baxt (5) in 1878 reported that stimulation of an accelerator nerve chiefly reduces the phase of systole. His technical procedures were, however, crude and entirely unreliable. Contrary effects on the duration of systole were reported from the stimulation of the vagus nerve by Klug (6) in 1881, and by MacWilliams (7) in 1888. The inability to decide questions of this nature, by their methods, is now obvious. The first experiments, therefore, that today would be regarded as accurate and at all decisive were made by Hürthle (8) in 1891. This investigator recorded the arterial pulse tracings with his membrane manometer and used the interval from the primary rise to the dicrotic notch as an index of the duration of systole. He found that the period of systole, so determined, is slightly abbreviated when cardiac acceleration is induced by stimulation of the accelerator nerves or when the vagi nerves are sectioned. Accelerator nerve stimulation, after sectioning of the vagi nerves, produces a marked decrease in the duration of systole; stimulation of the vagi, on the other hand, affects systole very slightly, but exerts its chief influence on the duration of diastole. In 1897 Frank (9) not only substantiated this work but reported, in addition, that by simultaneous stimulation of the vagi and accelerator nerves with suitably adjusted currents, it is possible to decrease the duration of systole even when the length of the diastolic phase is unaltered. In the comprehensive investigations of the accelerator and vagi nerve action, carried out by Reid Hunt (10) in 1899, can be found, among other data, the following observations: Section of the accelerator nerves causes a prolongation of both systole and diastole, the former being lengthened rather more than the latter. Stimulation of the accelerator nerves causes a shortening of both systole and diastole. Stimulation of a vagus nerve chiefly prolongs diastole, affecting systole relatively little. Under certain conditions, simultaneous stimulation of vagus and accelerator nerves produces a shortening of systole while diastole remains unaffected.

The conclusion that such results are not in accord with a mechanical regulation of the heart beat does not necessarily follow. Since the rate of systolic ejection diminishes toward the end of systole the duration of systole must by the law of "uniformity of behavior" become increasingly abridged as the cycles shorten more and more. Thus, in the volume curve reproduced in figure 1, a reduction in cycle length from 0.8 to 0.7 second entails a reduction in the ejection phase of systole from 0.215 to 0.21 second; while an equivalent reduction in the cycle length from 0.4 to 0.3 mathematically decreases the ejection phase from 0.175 to 0.15 second. Inasmuch as vagus section and vagus stimulation ordinarily do not alter the heart rate beyond ranges where slight variations might be expected, whereas accelerator stimulation quickens the beat so much that a more pronounced shortening of systole might be anticipated, it follows that *the mere demonstration that accelerator stimulation shortens the systole is proof neither of any specific influence of these nerves over ventricular contraction, nor does it prove that the heart deviates from a mechanical scheme. Only if it can be shown that the periods of systole during accelerator nerve stimulation vary materially from those which may be accounted for on the basis of volume curves, can any inference be drawn as to a selective action of the accelerator nerves on the ventricle.*

METHODS OF INVESTIGATION

In order to determine whether the lengths of systole and diastole during accelerator stimulation conform to or deviate from a mechanical regulation of the normal heart beat, we first established a plot of the theoretical systoles that should obtain at different cycle lengths and then compared, in the form of a plot, the actual systoles at different cycle lengths with these theoretical values.

Experimental procedures. In order to accomplish this it was necessary to determine accurately the duration of systole and diastole while the circulatory conditions were as nearly normal as possible. It was especially important, for example, to avoid opening the chest and the institution of artificial respiration—events which in themselves alter venous pressure relations considerably.

We therefore determined the systole and cycle lengths by optically recording the heart sounds by means of the direct sound recording capsules of Wiggers and Dean (11). The main vibrations of the first sound correspond to the first rise of intraventricular pressure while the first vibration of the second sound is synchronous with the incisura of the aortic pressure curve, events which mark the onset of systole and diastole respectively (12).

Dogs anesthetized with morphine and chloretone were used as experimental animals. The vagi and accelerator nerves together with the stellate ganglion were first prepared for section and stimulation, the latter being dissected without opening the thorax. The thorax was then shaved and a sound receiver adjusted over the apex region by an elastic band encircling the thorax. This receiver was connected with the sound recording capsules by a tube having an adjustable lateral opening. The vibrations of a 50 v. d. tuning fork were simultaneously recorded. In order to gauge the appropriate time for taking sound tracings on bromide paper, a carotid-pressure curve was continuously traced on a long paper kymograph.

From the optical records, the lengths of consecutive cycles and corresponding systoles were subsequently determined. This was done in about 3000 cycles recorded during many experiments on ten different dogs.

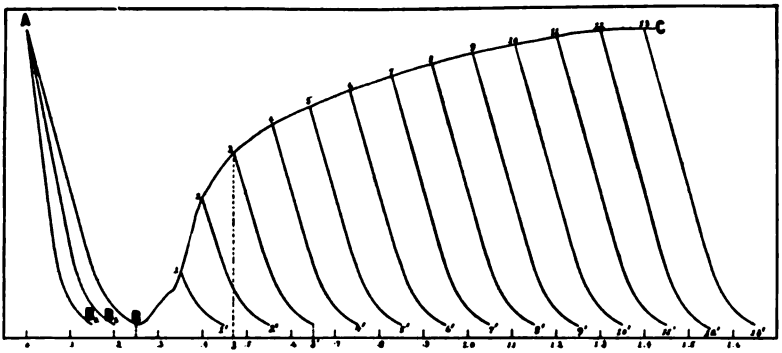

Fig. 1. (½ original size.) Diagram constructed for experiment C 207, determining the relation of systole and cycle length at various heart rates—also the method of adapting volume curves to vagal beats with different periods of systole. Shows arcs used when ejection phases *AB*, *AB'* and *AB*² are equal to 0.25, 0.20 and 0.15 second respectively. *B-1'*, *B-2'*, *B-3'*, etc., indicate duration of cycle. *1-1'* *2-2'*, *3-3'*, etc., the period of systole. Abscissa = 0.1 second. Detailed description in text.

Methods of constructing a curve expressing the theoretical duration of systoles at different cycle lengths. In order to obtain a curve of the theoretical systoles at all heart cycles according to Henderson's mechanical conception of cardiac control, it was first necessary to plot a theoretical volume curve for each animal. This construction, however, is beset with a number of difficulties which, we believe, are not insurmountable. We started out according to a very simple plan: On large sized coördinate paper, a volume curve similar to that plotted as a standard by Henderson (13) was laid off. A reduced reproduction *with coördinates omitted*, is shown in figure 1 (*A*, *B*, *C*). At varying points, e.g., at *2*, *3*, *4*, *5*, etc., arcs of the standard ejection curve *A B* were drawn. The abscissal distances *B–1′*, *B–2′*, etc., then denote the duration of the cycle and the abscissal distances *1–1′*, *2–2′*, etc., measure the corresponding systole lengths. These intervals can obviously be readily and accurately determined on large coördinate paper for a consecutive range of cycles. In figure 1, one such cycle *B–3′* and its corresponding systole *3–3′* is indicated by dotted lines. So far the process is not dissimilar to that employed by Henderson except that the relation of systole to each cycle length was determined in cycle lengths differing by 0.1 second. This $\frac{\text{systole}}{\text{cycle}}$ ratio will hereafter be referred to briefly as the s/c ratio.

The data so obtained were plotted by dots on coördinate paper, as shown in figure 4, the ordinates representing the duration of systole, the abscissae, the cycle lengths. By connecting these data by lines a curve s/c is obtained from which the theoretical s/c ratio at any heart rate can be derived.

It soon became obvious, however, that such a theoretical curve of s/c ratios could not be applied to different animals, inasmuch as certain variable factors were not taken account of. *In the first place* we found that the duration of long vagal systoles varied from 0.22 to 0.32 second in different animals with corresponding variations at more rapid rates. It therefore became necessary to construct for each animal a separate hypothetical volume curve based on its vagal systole and from it to derive a curve of s/c ratios applicable to that animal.

This we did after the following manner: The duration of systole was first determined during a long vagus beat occurring after slowing had been established for some time. Inasmuch as only the interval of systolic ejection and not the total period of systole is concerned in the construction of the volume curve, an interval of 0.05 second was deducted from the vagal systole for the isometric period.[1]

The resulting interval of systolic ejection was then laid off on the abscissae of large-sized coördinate paper and an arc having the same contour as that given in Henderson's standard curve was drawn to fill this time. Thus, in figure 1, the arcs *AB*, *AB*¹ and *AB*² have the same

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[1] This we believe to be allowable for, according to the investigations of Hürthle (8), de Heer (14), Garten (15) and Frank (16), this is an average period which is not affected in length by such changes in the circulation as occurred during the course of our experimentation. Even if this period does vary slightly in different dogs, no significant error can be introdued since the same figure was again added before the plot of s/c ratios was made.

conformation but correspond to ejection periods of 0.25, 0.20 and 0.15 second, respectively. In this particular instance, the arc *AB*, with a duration of 0.25 second was subsequently used as a pattern for the smaller segments *1–1′*, *2–2′*, *3–3′*, etc. It is obvious that if the systolic ejection time were either 0.15 or 0.20 second, the arcs *AB′* or AB^2 must be used as a pattern for the smaller segments.

The further criticism may be anticipated that belief in a "uniformity of behavior" law does not necessitate the assumption that hearts of different animals with the same systole lengths necessarily have the same contour of ejection curve. According to Henderson's (4) results, however, volume curves taken under normal conditions show that the curve of systolic discharge is a straight line at all points except at the extreme lower end. A variation which could occur only at this place would therefore affect the duration of only the very shortest cycles, e.g., *1–1′* in figure 1. Since cycles of such short duration were never obtainable in any of our experiments, however, it is clear that this criticism is of no practical importance.

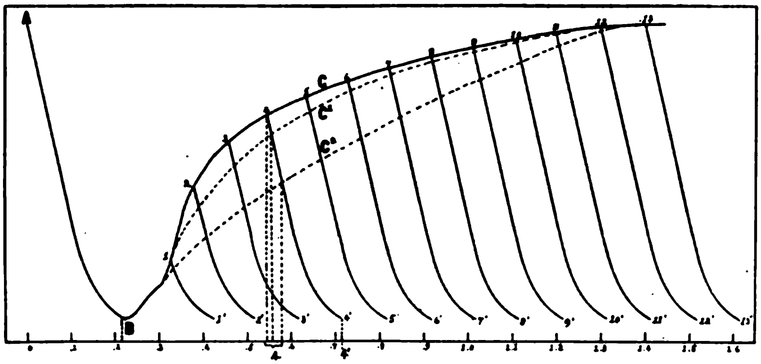

Fig. 2. (½ original size.) Diagram constructed for experiment C 210, showing volume curve with ejection phase of 0.215 second and three possible diastolic filling curves *C*, *C′* and C^2. The effect of diastolic filling rate on the length of systole is indicated by the systoles *4–4′*. Abscissa = 0.1 second. Detailed description in text.

A second practical difficulty arose, however, in the correct projection of the diastolic filling curve. Even though all possible precautions were taken to maintain an effective venous pressure sufficient, according to Henderson and Barringer (4), to insure maximal filling, it would not be in disagreement with the idea of superimposable beats to suppose that the filling curves of different animals are dissimilar. Indeed it is quite conceivable that the heart within the intact thorax has a filling curve quite different from any capable of registration by oncometric methods. Such differences in filling, however, will affect considerably the relation of systole to cycle length at different heart rates. This is illustrated in figure 2 where one may suppose the heart to be filled according to any of three curves *C*, *C′* or *C²*. Reference to the dotted lines bracketed as *4* in figure 2 or to the three corresponding plotted curves of s/c ratios in figure 3, indicates that the theoretical curves depend fundamentally on the unknown character of the filling curve.

Confronted with the necessity of having some gauge as to the type of filling occurring in each animal's heart, we selected that line of s/c ratios which most nearly coincided with the long vagal beats and the normal beats of the animal.

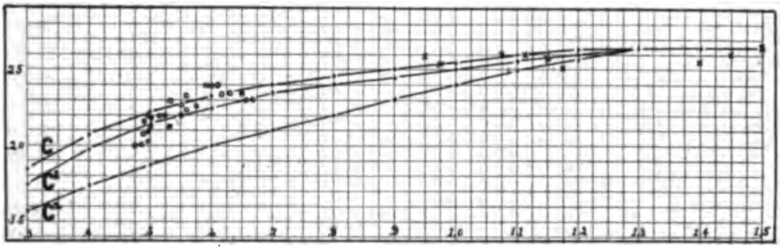

Fig. 3. Three curves showing relation of systole to cycle length at different heart rates when the rate of diastolic filling differs. Constructed from data of table 1 and volume curves of figure 2. *C*, *C′* and *C²* are corresponding curves. Abscissae represent cycle length; ordinates, systole lengths, in seconds. Circles, normal s/c ratios; crosses, s/c ratios of vagal beats. Detailed description in text.

The entire method of deriving our theoretical curve of s/c ratios, thus outlined in principle, may be further clarified by following the consecutive steps in a typical experiment:

In experiment C 210, stimulation of the right vagus caused a slowing of the ventricles from which a cycle having a period of 1.55 second and a systolic period of 0.265 second was selected. Deducting 0.05 second for the probable isometric interval, leaves an ejection phase of 0.215 second. This distance is laid off on coördinate paper and an arc *AB* drawn. A reduced figure with coördinates omitted for reasons pertaining to reproduction is shown in figure 2. In this case, three possible filling curves, *C*, *C′* and C² are drawn. By inscribing arcs of the ejection curve *A B*, at intervals of 0.1 second from points *1*, *2*, *3*, *4* and so forth, and measuring the horizontal distances between *1–1′*, *2–2′*, *3–3′*, for each type of filling curve (illustrated by dotted lines bracketed as *4*) three possible durations of the ejection phase for each cycle length are obtained. Now adding again 0.05 second previously deducted gives the theoretical systoles for each cycle under three conditions of ventricular filling. This is shown in the following table:

TABLE 1

DURATION OF CYCLE	DURATION OF THEORETICAL EJECTION PHASE IN FIGURE 2			DURATION OF TOTAL SYSTOLE ACCORDING TO FIGURE 3		
	Curve C	Curve C′	Curve C^2	Curve C	Curve C′	Curve C^2
0.2	9.0	9.0	8.5	14.0	14.0	13.5
0.3	13.5	12.5	10.75	18.5	17.5	15.75
0.4	15.75	14.5	12.0	20.75	19.5	17.0
0.5	17.25	16.5	13.75	22.25	21.5	18.75
0.6	18.0	17.5	15.0	23.0	22.5	20.0
0.7	19.0	18.5	16.0	24.0	23.5	21.0
0.8	19.5	19.0	17.0	24.5	24.0	22.0
0.9	20.0	19.5	18.0	25.0	24.5	23.0
1.0	20.5	20.0	19.0	25.5	25.0	24.0
1.1	21.0	20.5	20.0	26.0	25.5	25.0
1.2	21.5	21.0	20.7	26.5	26.0	25.7
1.3	21.5	21.5	21.25	26.5	26.5	26.25
1.4	21.5	21.5	21.5	26.5	26.5	26.5
1.5	21.5	21.5	21.5	26.5	26.5	26.5

Plotting these theoretical systoles in relation to the cycle, as in figure 3, the three curves *C*, *C′* and C^2 are obtained. If now we plot as small circles the actual s/c ratios found during the natural heart cycles of the animal, it will be seen that they follow the line *C′* most exactly. This line is therefore adopted as the theoretical line of s/c ratios for other comparisons. In a similar way, a line of s/c ratios was derived for all experiments and in the rest of the plots the line selected is alone reproduced.

It is of interest to add that, according to these analyses, we found that the hearts of our animals followed a type of filling not dissimilar to that described by Henderson as typical for the normal heart. In this instance we believe, for example, that the volume curve of the ventricle corresponds to the line *C′* in figure 2.

EXPERIMENTAL RESULTS

Comparison of the actual s/c ratios during accelerator nerve stimulation with the theoretical values at corresponding heart rates. After the theoretical curve of s/c ratios had been thus determined for each animal, we plotted the actual s/c ratios obtained under different experimental conditions in relation to it, especial attention, of course, being directed to the influence of the accelerator nerves. As the results require detailed presentation, an analysis of three experiments, typical of all cases, is appended.

Experiment C 207 (fig. 4). The theoretical s/c ratio curve selected as applying to this animal's heart is shown as a solid line (s/c) connecting dots at 0.1 second intervals. The small circles close to the line show normal s/c relations. The right stellate ganglion was stimulated first, but we shall defer the discussion of this effect. Then both vagi nerves were sectioned. The s/c ratios of 13 measured cycles are indicated by crosses of group *I.* It is evident that when vagus control is suddenly removed and the accelerator control alone remains, the s/c ratio is below that of the theoretical line. Within a few minutes, however, the duration of systole increases again, as shown by the crosses of group *II,* which represent measurements of 13 cycles ten minutes after vagotomy. The left vagus nerve was then stimulated but, with the exception of a few beats, the cycles were so long that they were omitted from this plot for reasons of reproduction. It may be noted, however, that the systoles following immediately after stimulation began were slightly below the theoretical line, while those occur-

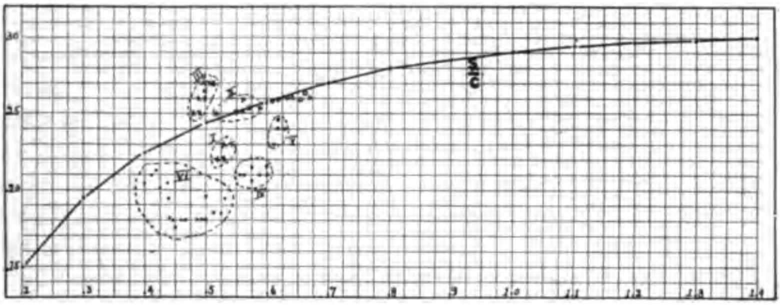

Fig. 4. Plot from experiment C 207, showing relation of actual s/c ratios to the theoretical curve. Abscissae, cycle lengths; ordinates, systole lengths, in seconds. Detailed description in text.

ring later coincided with it. Immediately following this stimulation, 12 measured cycles showed s/c ratios represented by the crosses of group *III*. The obvious after-effect seems to be a slight increase in the heart rate during which the systole lengths are somewhat longer than the theoretical curve calls for. Such results indicate that perhaps vagus stimulation is not entirely without an influence on ventricular systole,—whether direct or indirect we are not able to say. A detailed discussion of this question is, however, not within the province of the present communication.

We may now return to the effects of accelerator nerve stimulation. The dots of group *IV* represent the s/c ratios of 17 measured cycles recorded during accelerator stimulation while the vagus nerves were still intact. The dots of group *V* show the s/c ratios of 14 measurements shortly after the cessation of stimulation. The dots of group *VI*, representing 32 measured cycles indicate the greater effect of accelerator stimulation when the vagi nerves had been divided.

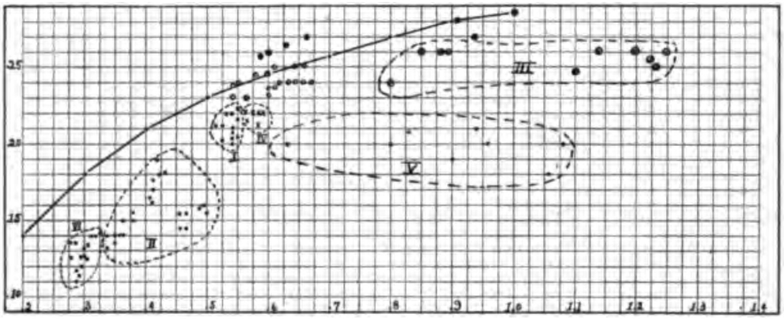

Fig. 5. Plot of data from experiment C 209, showing relation of actual s/c ratios to theoretical curve. Abscissae, cycle lengths; ordinates, systole lengths in seconds. Description in text.

The data plotted in relation to the theoretical s/c ratios make it obvious without further comment that the stimulation of the accelerator nerves reduces systole a great deal more than can be accounted for by a mechanical abbreviation of the systolic portion of the volume curve.

Experiment C 209 (*fig. 5*). The line of theoretical s/c ratios in this experiment was derived from a vagus beat having a cycle length of 0.98 seconds and a systole length of 0.285. The small circles arranging themselves around the theoretical line are normal cycles representative of 40 measured beats. The large dots represent ratios occurring during vagus excitation. The small dots of group *I* indicate the duration of systole during mild stimulation of the stellate ganglion; those of group *II*, the effect of exciting the ganglion with a stronger current. In both instances the vagus nerves were intact. It is evident that a reduction of s/c ratios below the theoretical value occurs.

In order to determine the effect of accelerator stimulation on the s/c ratio when cycles of longer duration occurred, we tested the effect of simultaneous stimulation of the accelerator and vagus nerves. This could, of course, be accomplished by simultaneous electrical stimulation of these nerves. We found it, however, more expedient to induce the vagus stimulation through chemical means. Pituitary extract when injected, produces such a stimulation in some animals as was fortunately the case in this experiment.

The dotted circles of group *III*, arranging themselves somewhat below the theoretical line, show the duration of systole during such pituitary slowing. That this slowing is at least predominantly due to vagus stimulation, is evidenced by the fact that subsequent section of the vagi restores the normal rate and duration of systole. This is shown by the crosses of group *IV* which represent the systoles of 18 measured cycles after the vagi nerves were cut and while pituitrin was still acting.

While the heart rate remained slow due to the pituitary extract, the stellate ganglion was again stimulated. Although this caused some increase in rate, the heart rate did not equal that natural to the animal. The s/c ratio decreased not only far below the theoretical ratios for such cycles, but also far below the s/c ratios of other much shorter cycles. This is evident on comparing the dots of group *V*, representing 9 measurements of beats occurring during accelerator nerve stimulation, either with the dots of group *I*, with the crosses of group *IV* or with the circles representing normal s/c ratios. Finally, after the vagi had been divided and s/c ratios represented by the crosses of group *IV* had been attained, the stellate ganglion was again stimulated. The results of 27 measurements are shown by the dots of group *VI*.

These results indicate clearly that the s/c ratio is much reduced below the theoretical expectations by the influence of the accelerator nerves, not only at rapid, but at slower heart rates as well.

Experiment C 210 (fig. 6). In this experiment the curve of the theoretical s/c ratio was derived as analyzed in detail in an earlier portion of the paper. The small circles represent the actual s/c ratios of 60 normal beats. The small circles in group *III* are representative of cycles following the injection of $\frac{1}{100}$ grain of atropine sulphate.

The dotted circles show the s/c ratios of 13 cycles obtained during the action of pituitary extract early in the experiment. Section of the vagi abolished this slowing and established a normal rate. Seven measurements of right vagus and 18 observations of left vagus stimulation are shown by crosses. Under all of these conditions of varying vagus activity the s/c ratio does not deviate in any pronounced fashion from the theoretical line.

On accelerator nerve stimulation, this conformity is no longer observed. The dots of group *I* represent measurements of 53 cycles during and immediately after accelerator nerve stimulation. The dots of group *II* show the results of 33 measurements of cycles obtained while the accelerator nerves were stimulated and the heart slowed by pituitary extract. With the exception of three dots in this group which, as a matter of fact, correspond to cycles at the very onset of stimulation, the s/c ratios are far below the theoretical line.

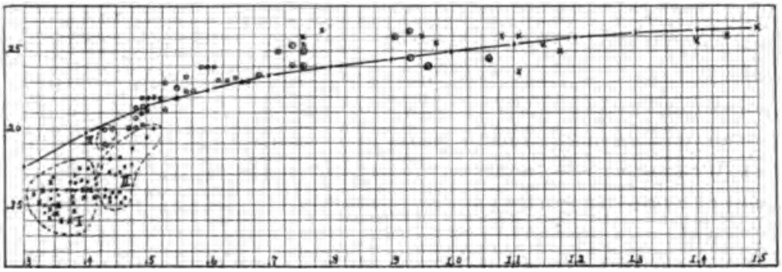

Fig. 6. Plot of data from from experiment C 210, showing relation of actual s/c ratios to theoretical curve. Abscissae, cycle lengths; ordinates, systole lengths, in seconds. Description in text.

Finally, when the vagi nerves had been sectioned, 3 cc. of a 1:50,000 solution of epinephrin, which presumably also affects the sympathetic endings in the heart, were injected. The crosses included in group *I* illustrate the s/c ratio of 44 rapid beats thus produced. It is obvious that epinephrin like accelerator stimulation acts to abbreviate systole much more than can be explained by a mechanical shortening of systole at these rates.

The mechanism of accelerator action. Discussion of results. The experiments above cited indicate clearly that, while under normal conditions changes in the duration of systole may conform reasonably to the changes anticipated if the ventricle beats according to a uniform plan, the abbreviation of systole induced through accelerator activity both at rapid and slower heart rates is in excess of that which may be accounted for by a mechanical shortening. In some way the accelerator nerves exert an influence on ventricular systole which can be termed specific. Before it may be assumed, however, that such an influence is exerted directly on the ventricular muscle and acts to abbreviate the contraction process, diligent inquiry must be made for the involvement of some possible mechanical mechanism.

The possibility suggests itself at once that the rapid heart action induced by accelerator stimulation causes an increase in the minute volume discharged which might conceivably operate to reduce the venous pressure and auricular filling. If this were the case, the rate of ventricular filling, especially in early diastole, would decrease and this might mechanically abbreviate systole. In other words, it is conceivable that under rapid heart action we would have an entirely different filling curve and that therefore the theoretically derived curve would no longer apply. Unfortunately, we did not follow the venous pressures throughout the experiment nor do such observations during accelerator stimulation seem to have been reported. In the volume curves recorded by Henderson (4) during accelerator nerve stimulation, there is no such indication of reduced filling; indeed the rate of filling appears to us slightly increased. Such an explanation, however, could not account for the greatly abbreviated systoles occurring when the accelerator nerves affect beats maintained at or below the normal rate by simultaneous vagus action.

The further suggestion that the reduction of systole by accelerator stimulation is due to a shortening of the isometric period rather than the ejection phase, might be entertained were the reduction itself not frequently far greater than the entire isometric interval. By no plausible conception at present presenting itself can the effect of the accelerator nerves be explained otherwise than through a specific effect on the duration of muscular contraction itself.

This explanation is further substantiated by the lack of a fixed relation between the duration of systole and diastole when we follow them from beat to beat during increased accelerator activity. Thus, accelerator nerve stimulation usually causes an immediate shortening of diastole while the period of systole shortens gradually and progressively as stimulation continues. In some experiments, systole continues to shorten even when diastole is again increasing. We have plotted experiments in which, during continued stimulation, diastole actually lengthened again so that its duration was greater than normal, yet systole continued at its shortest length. When stimulation ceases the systolic period usually remains shortened for as much as 20 seconds; a shortened diastole, on the contrary, at once begins to increase in length. The general trend of such experiments is shown in an abbreviated plot in figure 7. At *A*, a few consecutive normal cycles are indicated. At *B*, the 20th and subsequent beats during accelerator stimulation are shown. A moderate decrease in systole and a marked decrease in diastole are evident at this time. As stimulation continues (*B-C*), systole decreases more than diastole. Between *C* and *D*, 25 beats are omitted. While accelerator stimulation continues at *D*, the length of systole remains practically unaltered while the period of diastole has recovered considerably. At *E*, stimulation ceased. Between *E* and *F*, 6 beats were omitted. By this time, *F*, diastole has regained its normal length, but systole continues shortened. *G* is a later normal control.

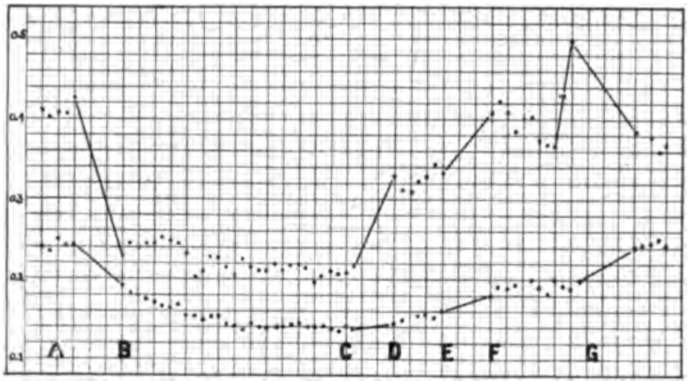

Fig. 7. Plot from experiment C 209 II, showing effect of accelerator nerve stimulation on duration of systole and diastole in consecutive heart beats, each represented by a dot. Upper plot, duration of diastole; lower curve, systole; ordinates represent time in seconds. Description in text.

Another illustration is shown in the case of epinephrin stimulation in figure 8. The plot starts with a few normal control data. At *A*, the systole and diastole after the 20th beat following epinephrin injection are plotted. Both vagi nerves had been severed and a previous dose of $\frac{1}{100}$ grain of atropine sulphate had been administered. In this instance, diastole first lengthens while systole progressively decreases (*A B*). Between *B* and *C*, 45 beats are omitted and by that time systole has again begun to lengthen while diastole decreases further. This again demonstrates the lack of a fixed relation between systole and diastole when the accelerator endings are stimulated.

In view of these facts and since cardiac acceleration under normal conditions is undoubtedly often due to accelerator nerve activity, the hypothesis that the ventricle normally beats according to a "uniformity of behavior" law at these rapid rates should be submitted to further experimentation.

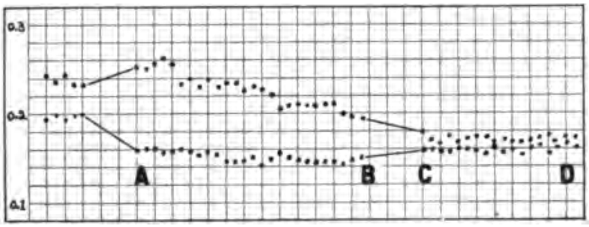

Fig. 8. Plot from experiment C 210 VII, showing effect of epinephrin on duration of systole and diastole in consecutive heart beats, each represented by a dot. Upper plot, duration of diastole; lower curve, systole; ordinates represent time in seconds. Description in text.

SUMMARY AND CONCLUSIONS

Although it had been shown by previous investigators that stimulation of the accelerator nerves causes a marked reduction in the duration of systole, it had not been demonstrated that this reduction was greater than could be accounted for on Henderson's law of "uniformity of behavior," and consequently no clear demonstration existed of any specific effect on the ventricular musculature.

By determining the duration of systole as well as the cycle length in a slow vagal beat, we found it possible to construct a probable volume curve for each animal and from it to derive a plot of the theoretical relation that should exist between cycle and systole lengths at any heart rate if the heart, during changing nervous action, beats according to a uniform law.

The actual systole and cycle lengths were determined from the recorded heart sounds during a wide range of heart rates obtained through vagus sectioning, vagus stimulation and accelerator excitation and these values were then plotted on coördinate paper in relation to the theoretically constructed curve of $\frac{\text{systole}}{\text{cycle}}$ ratios.

Upon doing this, it was found that while the actual $\frac{\text{systole}}{\text{cycle}}$ ratios obtained during normal conditions and during vagus stimulation coincided reasonably well with the theoretically derived values, *the length of systole during accelerator stimulation and during the action of epinephrin were markedly less than those indicated by the theoretical curve.* Inasmuch as this occurred whether the heart rate was actually increased or maintained slow by the simultaneous action of pituitary extract, it is difficult to refer this to any mechanical effect on the venous pressure and ventricular filling.

Consequently, the conclusions are reached *a*, that the accelerator nerves have a specific effect on the ventricular musculature which operates to reduce the contraction period; and *b*, that, in view of these observations, the hypothesis that under normal conditions the ventricle operates according to a uniform mechanical law, should be subjected to further investigation.

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GASTRIC RESPONSE TO FOODS[1]

XIII. The Influence of Sugars and Candies on Gastric Secretion

RAYMOND J. MILLER, OLAF BERGEIM, MARTIN E. REHFUSS
AND PHILIP B. HAWK

From the Laboratory of Physiological Chemistry of Jefferson Medical College, Philadelphia

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The widespread use in the diet of large quantities of refined sugars and candies is a comparatively modern development. Because the races of men have lived for ages without general access to sugars in concentrated form, a question has naturally arisen as to whether the use of such foods may not in some instances give rise to harmful effects. It was perhaps natural also that the rapid development of the glucose industry should bring forth opponents and proponents of its wide use in cooking and in confections.

It is not necessary at this date to refute statements with reference to the alleged harmful character of cane sugar or glucose per se, inasmuch as it is well known that all digestible carbohydrates are absorbed from the intestine in the form of simple sugars and in the main as glucose formed from the starch of foods.

Certain objections to the use of sweets must, however, be considered. It cannot be denied that the eating of candies before meals decreases the appetite for other foods in general and that thus, particularly in the case of children, the intake of foods containing essential proteins, vitamines and inorganic salts may be reduced below the optimum requirements for growth. Purified sugars can obviously furnish but the single dietary essential, carbohydrate. This depression of appetite may be associated with the rapid absorption which sugars undergo in the intestine as well as with the depression of gastric secretion which is indicated by data presented in this paper. Such work as has been carried out on the assimilation of carbohydrates as measured by the rise of blood and urinary sugar following their ingestion indicates that pure sugars are absorbed sooner from the intestine than the glucose formed from starch ingested (1). The assimilatory power of the normal human body for glucose would not appear, however, to be readily overtaxed.

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[1] The expenses of this investigation were defrayed from funds furnished by Mrs. M. H. Henderson, The Curtis Publishing Company and Doctor L. M. Halsey.

It is known that in diabetes the weakened assimilatory function of the system for carbohydrate is still further impaired by the ingestion of large amounts of such food, and it might be supposed that habitual, long-continued use of many sweets might lead to the aggravation of a diabetic tendency which might not otherwise manifest itself. Such a suggestion has been made, but there is little concrete evidence to support it.

Sugars and candies, on the other hand, are particularly suited to furnish to the body, in convenient form, an additional quota of readily assimilable energy. Thus it has been pointed out that a single caramel may furnish 45 calories or sufficient energy for a mile walk (2), and that other confections yield similar amounts of energy. The view that these preparations are of negligible food value must, therefore, be discarded. Surely there is a physiological basis for the candy craving of children which cannot be disregarded, unless energy in abundance is furnished by other foods, nor does there seem to be any good reason for replacing more of the food carbohydrate by fats (except those high in vitamine) which are less readily assimilated and for which children generally have less desire.

In the cases of gastro-intestinal derangements more common in adult life, the influence of diet on the secretory, motor and fermentative processes of the digestive tract may overbalance considerations of energy value.

In the present paper we have endeavored to determine the influence of certain sugars, candies and other confections on the secretory and motor responses of the stomachs of normal adults.

The experiments were carried out on normal medical students and members of the staff of the department. They reported about nine o'clock in the morning, and any residuums which were present were removed from the stomach. The sugar solutions or candies were then given and samples of stomach contents removed at 15-minute intervals until the stomach was empty. Free and total acidities, pepsin, trypsin and amino acid nitrogen were determined by methods previously described (3).

The response of the stomach was studied following the ingestion of cream candies, hard candies, chewing candies, fresh and stale candies, chocolate and candy combinations. Inasmuch as most sweets enter the stomach essentially as sugar solutions, a preliminary study was made of the influence of concentrated and dilute solutions of cane sugar and glucose.

The response of the stomach to dilute and concentrated solutions of sucrose, glucose and maple sugar. Nine experiments were made on dilute and concentrated sugar solutions. The results of these experiments are charted in figures 1 to 9. One subject was given 250 cc. portions of 4 per cent glucose and cane sugar solutions; another subject, 150 cc. portions of 6 per cent glucose and cane sugar solutions, the total amount of sugar given in each case being about 10 grams. The same evacuation time (1 hour and 45 minutes) was obtained in each of the four experiments, and the acid responses were very similar.

No distinction could, therefore, be made between the responses of the stomach to dilute solutions of glucose and cane sugar nor, as the curves show, could there have been any distinct depression of gastric secretion by the dilute sugar solutions in the quantities given.

Maple sugar in dilute solution was given to the same subjects and was found to leave the stomach in from 45 minutes to an hour and 15 minutes without distinct depression of gastric secretion. The fact that this solution left sooner than the cane sugar or glucose may have had some relation to the more pleasant taste of the maple sugar. The cases are not quite comparable, however, as the glucose and cane sugar solutions were given without removing residuums.

Concentrated sugar solutions were given the men who had previously received dilute solutions. One subject was given 100 grams each of glucose and cane sugar in 59 per cent solutions. The other subject was given 100 grams of glucose in 40 per cent solution. Such solutions remained in the stomach from one-half to an hour longer than similar volumes of the dilute sugar solutions. In the case of the glucose solutions, the secretion of gastric acid was markedly depressed for an hour and a half or until much of the glucose had left the stomach. The secretion of pepsin was inhibited also. Cane sugar in concentrated solution appeared to have somewhat more stimulatory power, but its evacuation was likewise delayed. It is possible that the sweeter taste of cane sugar or its less rapid absorption from the intestine may influence the response of the stomach to its concentrated solution, but more evidence on this point would be required. It is clear that con-

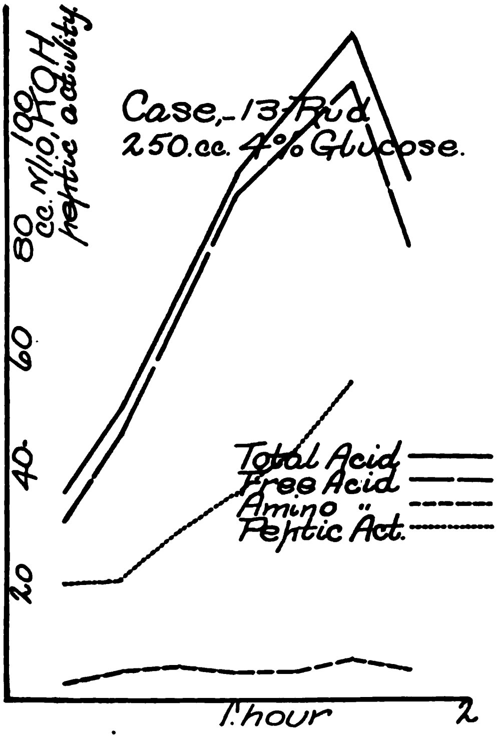
Case,- 13 Rud
250.cc. 4% Glucose.
cc. N/10, KOH.
peptic activity
20
40
60
80
100
1.hour
2
Total Acid
Free Acid
Amino "
Peptic Act.

FIG. 1

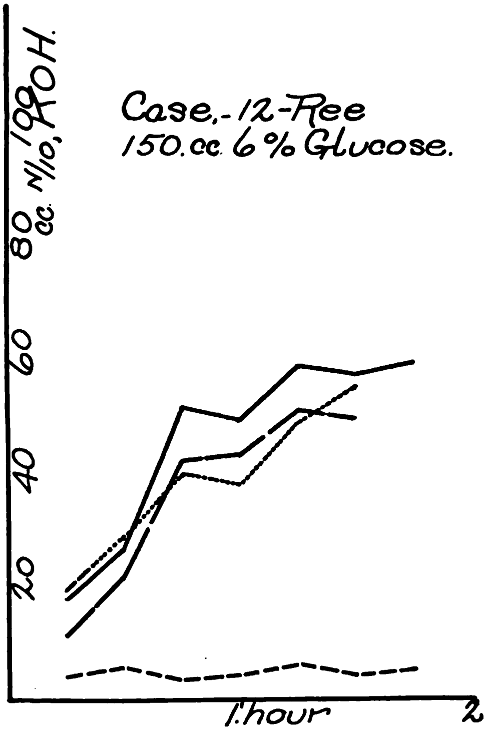
Case,- 12-Ree
150.cc. 6% Glucose.
cc. N/10, KOH.
20
40
60
80
100
1.hour
2

FIG. 2

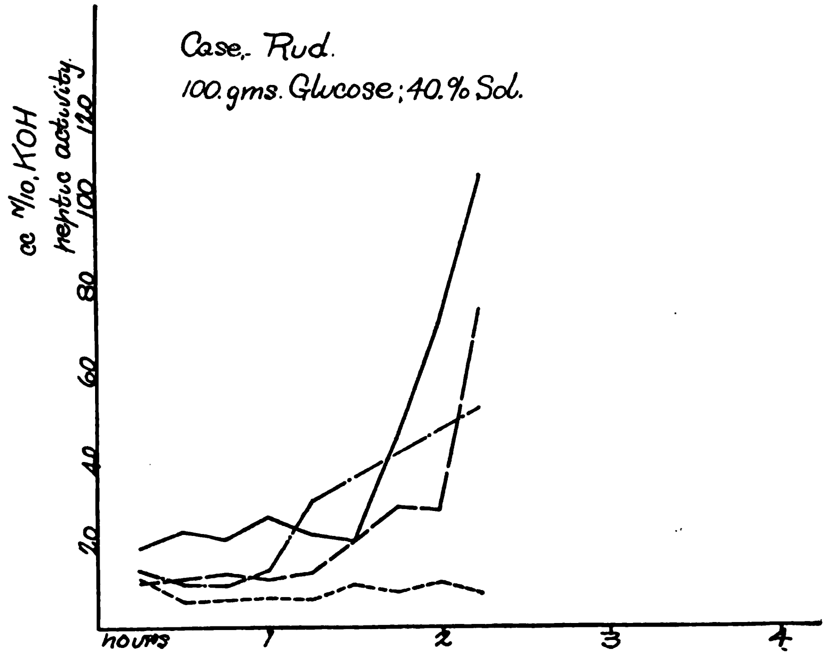
Case.- Rud.
100.gms. Glucose; 40.% Sol.
cc N/10, KOH
peptic activity
20
40
60
80
100
120
hours
1
2
3
4

FIG. 3

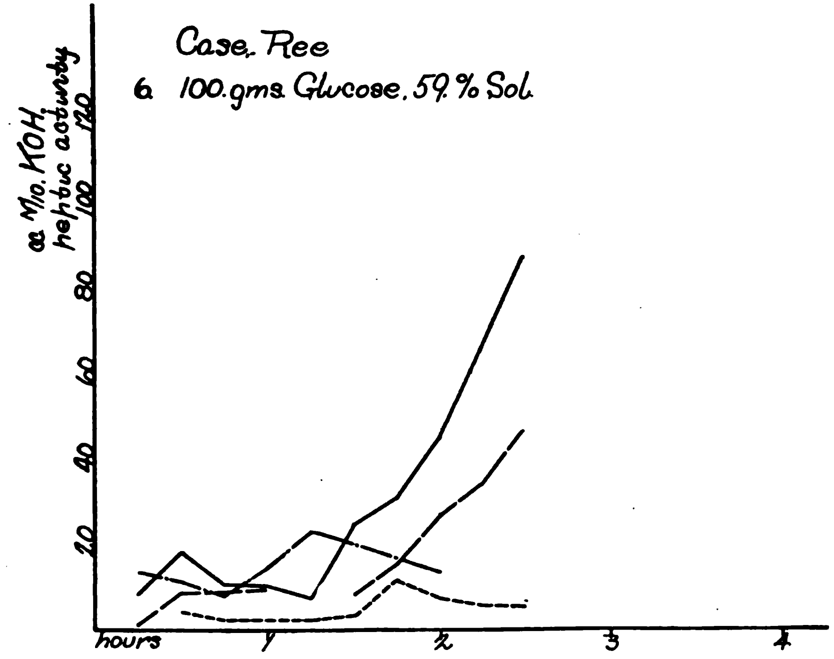
Case.- Ree
a 100.gms. Glucose. 59.% Sol.
cc N/10. KOH,
peptic activity
20
40
60
80
100
120
hours
1
2
3
4

FIG. 4

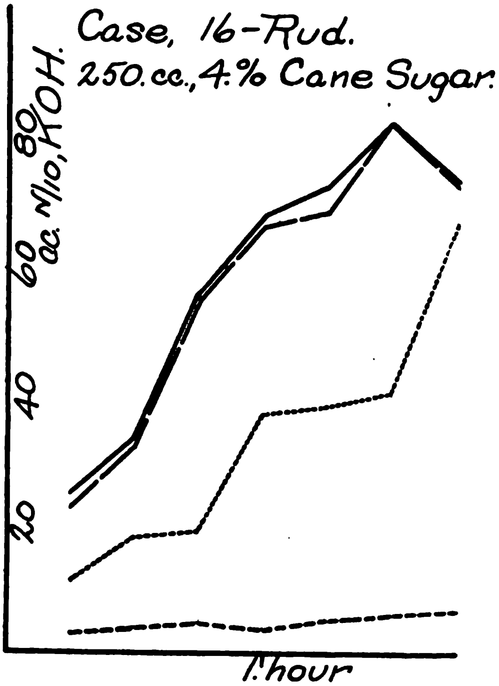
Case, 16-Rud.
250.cc., 4.% Cane Sugar.
cc. N/10, KOH.
80
60
40
20
1. hour

FIG. 5

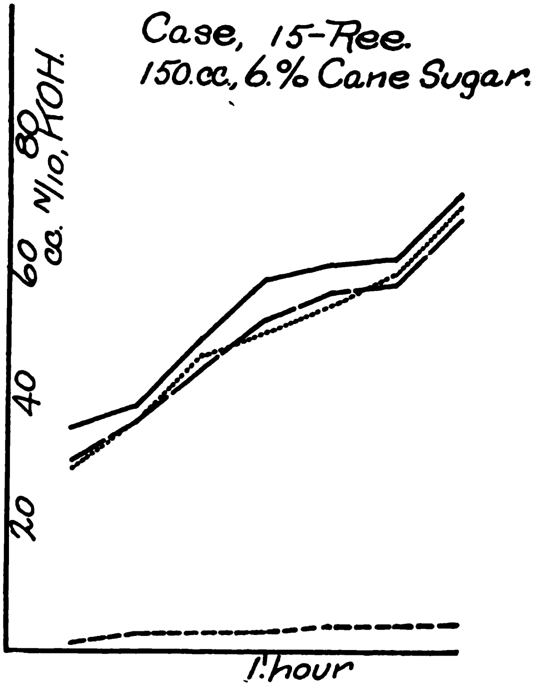
Case, 15-Ree.
150.cc., 6.% Cane Sugar.
cc. N/10, KOH.
80
60
40
20
1. hour

FIG. 6

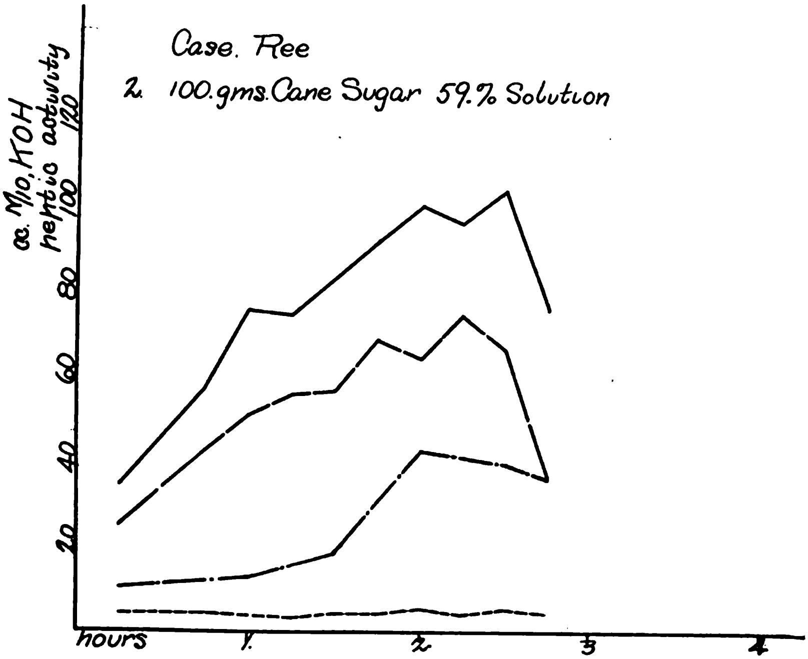
Case. Ree
2. 100.gms. Cane Sugar 59.% Solution
cc. M/10 KOH
peptic activity
120
100
80
60
40
20
hours
1.
2.
3
4

FIG. 7

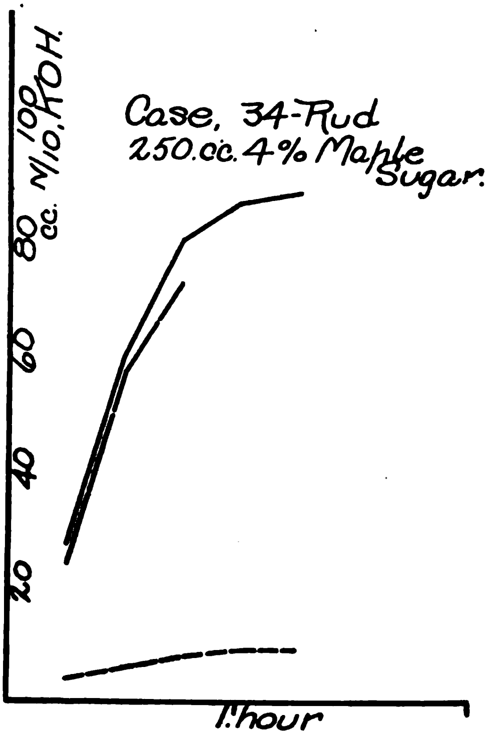
Case, 34-Rud
250.cc. 4% Maple Sugar.
cc. N/10. KOH.
100
80
60
40
20
1. hour

FIG. 8

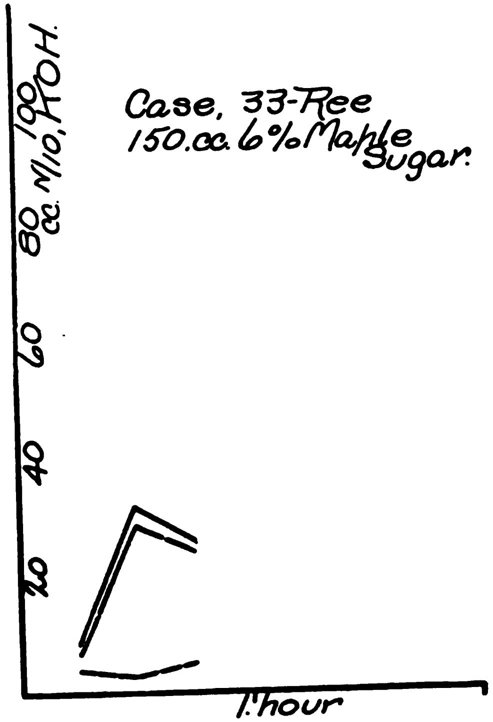
Case, 33-Ree
150.cc. 6% Maple Sugar.
cc. M/10, KOH.
100
80
60
40
20
1. hour

FIG. 9

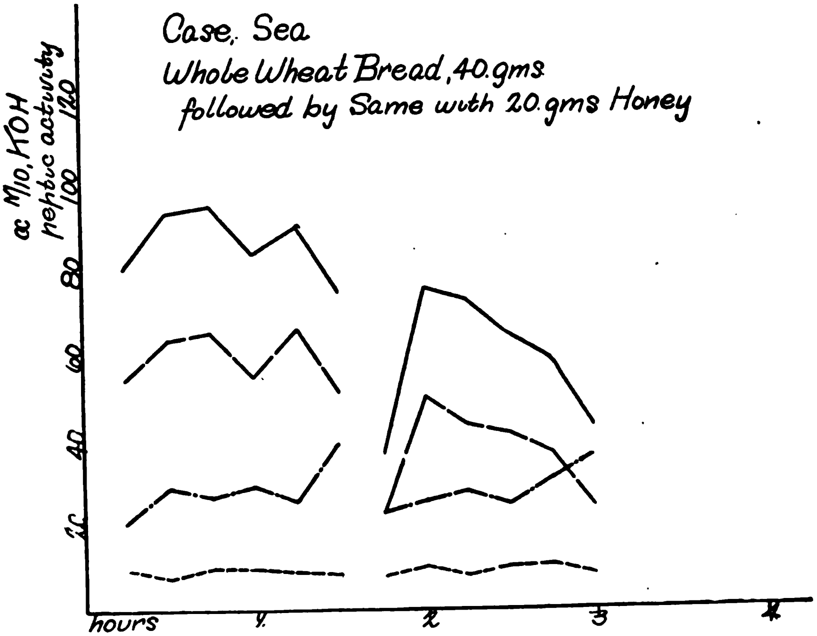
Case, Sea
Whole Wheat Bread, 40.gms
followed by Same with 20.gms Honey
cc M/10, KOH
peptic activity
120
100
80
60
40
20
hours
1.
2
3
4

FIG. 10

centrated sugar solutions markedly depress gastric secretion and delay evacuation.

Soft candies. Under this heading were included chocolate creams, fudge, bonbons and wafers. The contents alone of chocolate creams, and plain milk chocolate were also studied.

The interiors of chocolate creams (consisting mainly of glucose) were given in 100-gram portions to two subjects (see figs. 11 and 12). It will be noticed that gastric secretion was markedly inhibited and evacuation much delayed by the ingestion of this amount of cream candy which was given without water and formed a concentrated sugar solution in the stomach. The same depressing action was noted where soft creamy bonbons were given (see fig. 13). These contained somewhat more cane sugar and were more highly flavored than the creams previously tested. This may possibly have accounted for the slightly more rapid evacuation.

Soft creamy wafers of strawberry flavor were given to one subject, 100 grams of the candy being ingested. As might be expected, the gastric secretion was depressed by the large amount of sugar present. Evacuation, however, was completed in moderate time (1¾ hour), showing perhaps some influence of the fruit flavor on gastric motility.

Wafers of the same type but with strong peppermint flavor remained three-quarters of an hour longer in the stomach of this subject than did the strawberry wafers and gave rise to somewhat more acid secretion. The delayed evacuation may have been due to irritation of the duodenal mucosa by the oil of peppermint used as a flavoring agent.

Chocolate fudge remained in the stomach of one subject half an hour longer than a strawberry-flavored cream candy (see fig. 16). There was also a distinctly higher acid production in the case of fudge. Both of these effects must be related to the presence in the fudge of butter fat and chocolate.

That chocolate stimulates gastric secretion is indicated by our experiment in which milk chocolate was given (see fig. 17), an acidity twenty points higher being attained than in the case of creams. Milk constituents are probably responsible in part for this effect. Milk chocolate left the stomach in 2 hours or half an hour sooner than cream candy. It must, therefore, be considered as throwing less of a burden on the stomach than the latter.

Chocolate creams were compared in two cases with the contents of similar creams. Our best subject (see fig. 18) showed practically the same evacuation time for both but a somewhat higher acid develop-

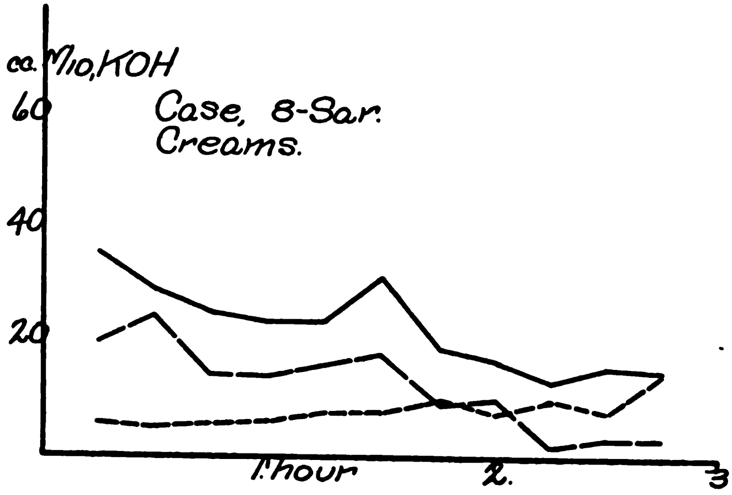
cc. N/10, KOH
60
40
20
Case, 8-Sar.
Creams.
1. hour
2.
3

FIG. 11

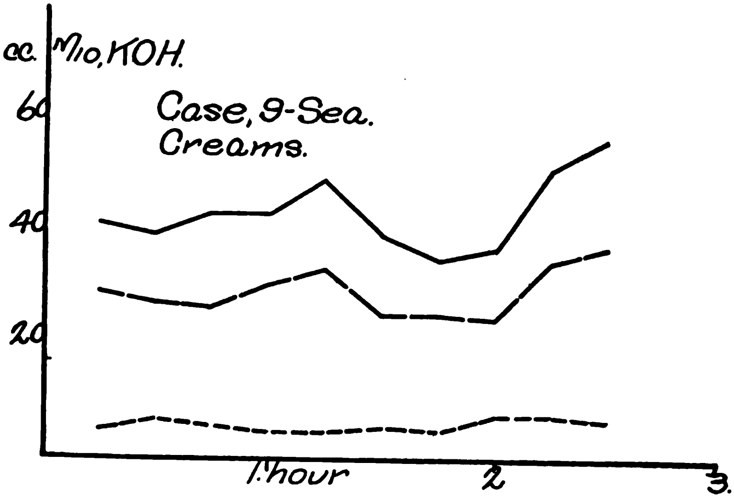
cc. N/10, KOH.
60
40
20
Case, 9-Sea.
Creams.
1. hour
2
3.

FIG. 12

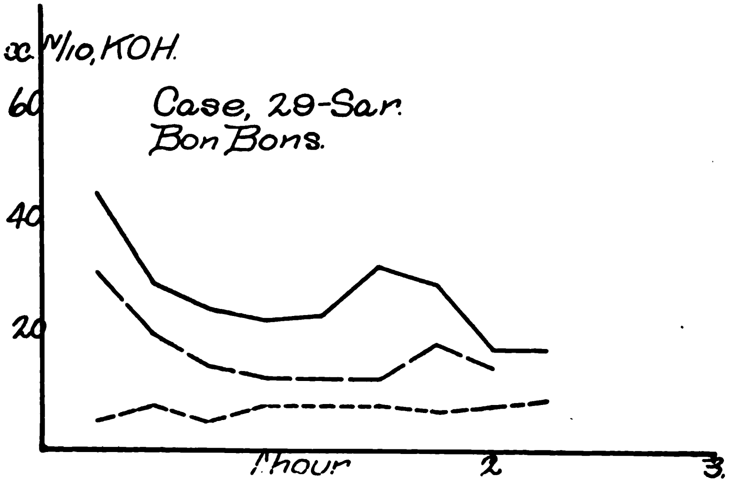
cc. N/10, KOH.
60
40
20
Case, 29-Sar.
Bon Bons.
1 hour
2
3.

FIG. 13

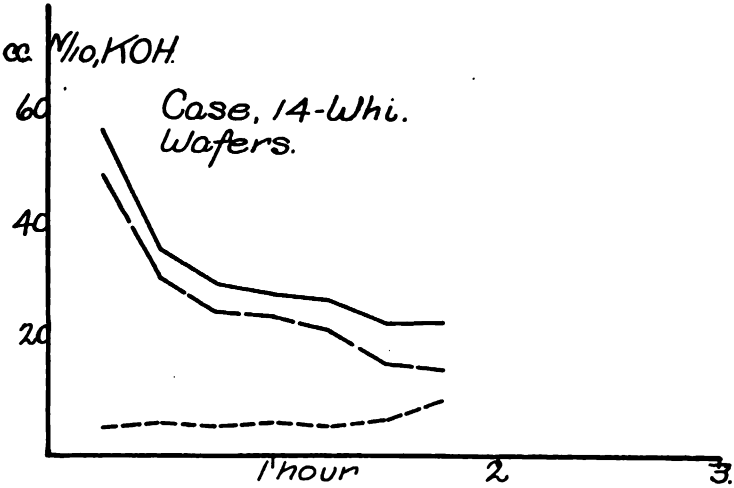
cc. N/10, KOH.
60
40
20
Case, 14-Whi.
Wafers.
1 hour
2
3.

FIG. 14

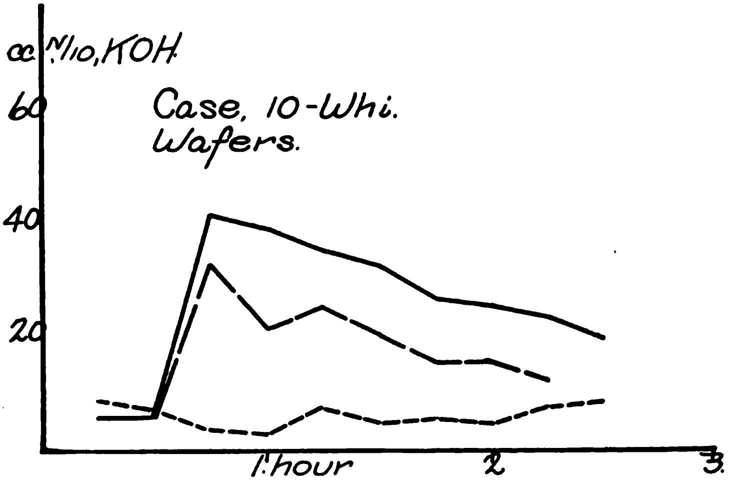
cc N/10, KOH
60
40
20
Case, 10-Whi.
Wafers.
1. hour
2
3.

FIG. 15

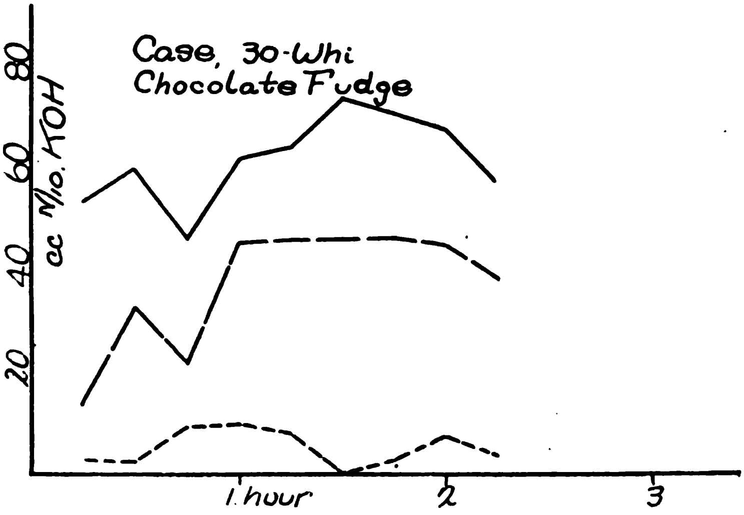
cc N/10. KOH
80
60
40
20
Case, 30-Whi
Chocolate Fudge
1. hour
2
3

FIG. 16

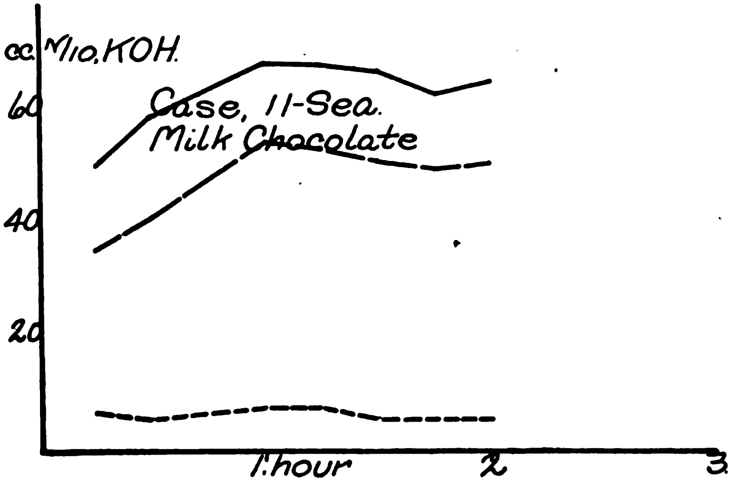
cc. N/10. KOH.
60
40
20
Case, 11-Sea.
Milk Chocolate
1. hour
2
3

FIG. 17

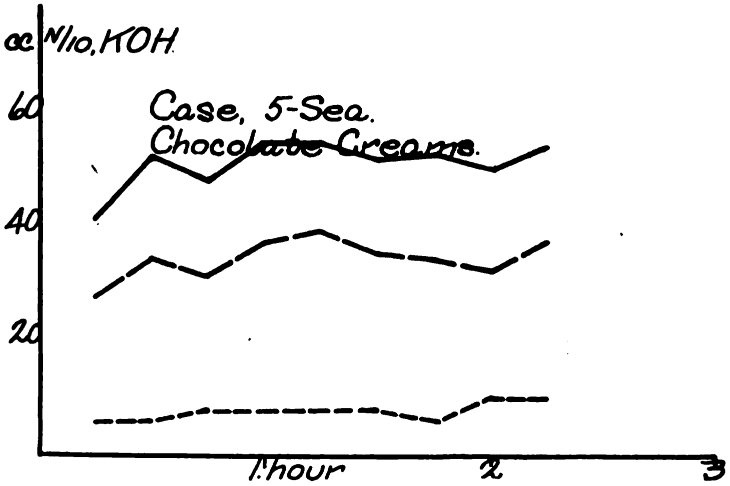
cc. N/10. KOH
60
40
20
Case, 5-Sea.
Chocolate Creams.
1. hour
2
3

FIG. 18

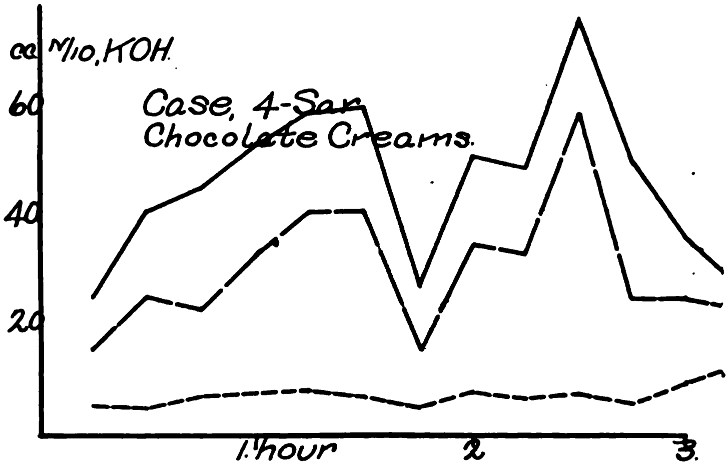

FIG. 19

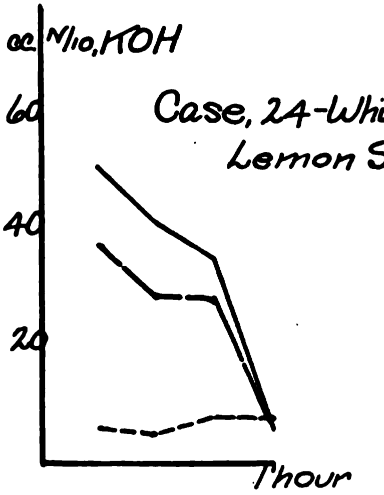

FIG. 20

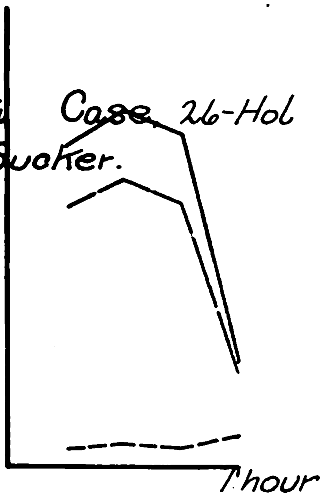

FIG. 21

ment on chocolate creams in agreement with our findings on chocolate alone. Our second subject also gave higher acidities on chocolate creams but evacuation was delayed due to considerable intestinal regurgitation characteristic of this subject. He was also given, for comparison, chocolate creams which were 8 months old and very stale and not appetizing. These showed delayed evacuation and high acidity as compared with cream candies, and it appears probable were less easily handled by the stomach than similar candy in the fresh condition. Interpretation is somewhat difficult on account of the excessive regurgitation of this subject.

Hard candies. Two men were permitted to suck continuously for 15 minutes on hard candies (lemon-flavored stick candy). The candies were weighed before and after, and it was found that one man succeeded in obtaining 15 grams of candy, the other only 7 grams. No water was taken by either during the course of the experiment so that the sugar must have entered the stomach as a solution of moderate concentration. In each case a slight gastric secretion of moderate acidity was developed, and the stomach was empty in an hour. Neither was there any distinct continued secretion afterward. It is clear that the burden placed on the stomach by sucking the hard candy was very much less than that produced by the liberal or moderate eating of cream candies.

Chewing candies. Caramels, salt water taffy and gum drops were the chewing candies studied (see figs. 22 to 25). Caramels gave rise to a much greater acid production than cream candies, although evacuation times were about the same. This acidity may have been due to the greater chewing psychic secretion as well as to direct stimulation by ingredients of the caramels other than sugar. At any rate the marked depressing action of pure sugar candies was not noted. The marked differences between free and total acidities were due largely to the action of the gastric acid on the phosphates of swallowed saliva.

In the case of gum drops experimental difficulties were met with as the gelatinous mass formed in the stomach clogged the aspiration tube. It is clear, however, that the gum drops left the stomach in moderate time and produced little acid stimulation and were thus handled without difficulty so far as the stomach was concerned.

Salt water taffy left the stomach of one subject sooner than caramels or creams but developed a much lower acidity than caramels. It may be that the chewing psychic secretion caused by eating caramels was greater due to their flavor more nearly approximating that of palatable food normally giving rise to a gastric stimulation.

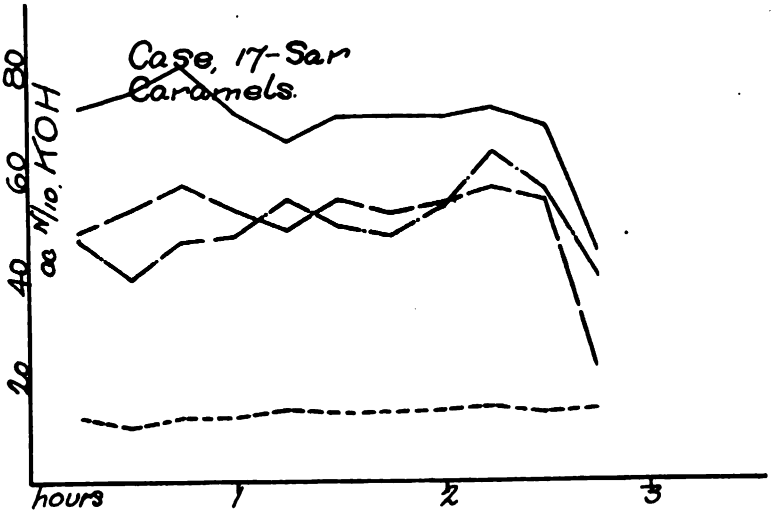
Case, 17-Sar
Caramels
cc N/10 KOH
80
60
40
20
hours
1
2
3

FIG. 22

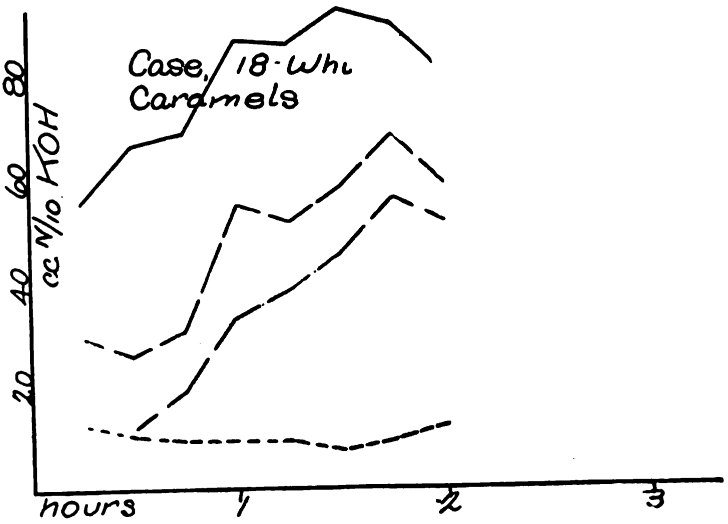
Case, 18-Whi
Caramels
cc N/10 KOH
80
60
40
20
hours
1
2
3

FIG. 23

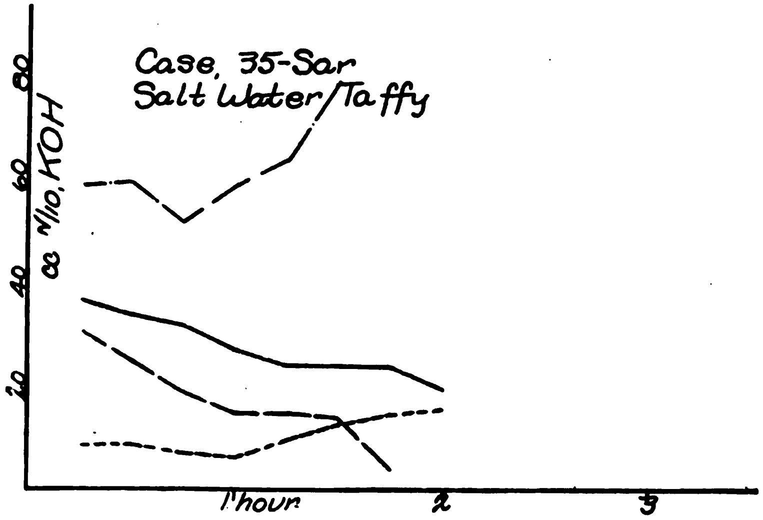
Case, 35-Sar
Salt Water/Taffy
cc N/10, KOH
80
60
40
20
1 hour
2
3

FIG. 24

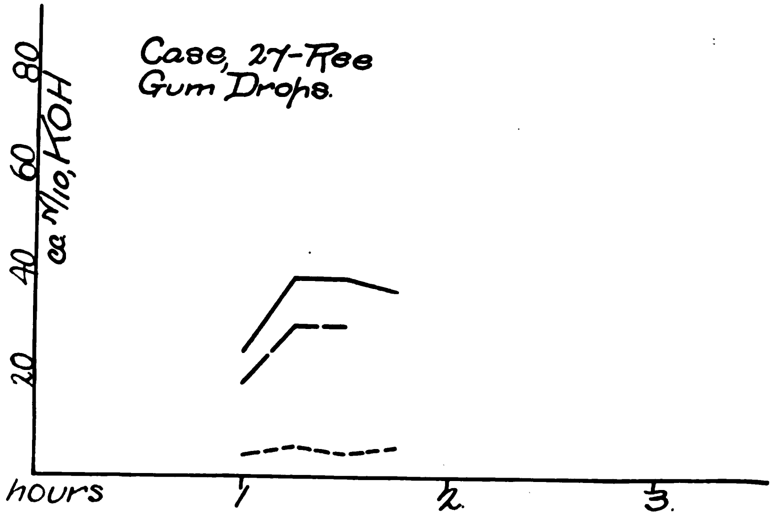
Case, 27-Ree
Gum Drops.
cc N/10, KOH
80
60
40
20
hours
1
2.
3.

FIG. 25

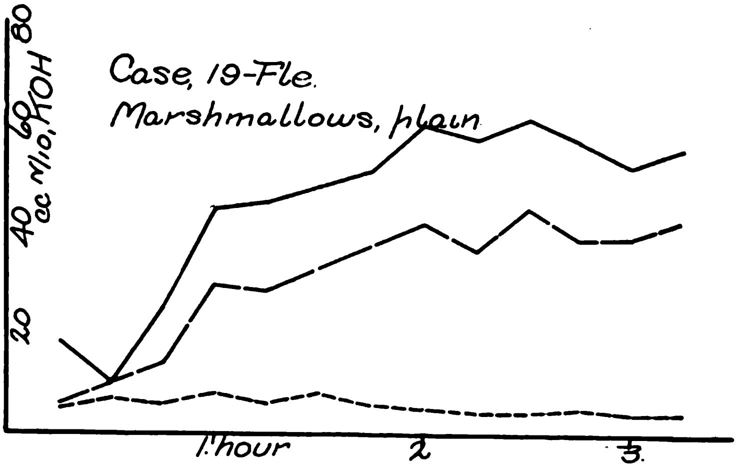
Case, 19-Fle.
Marshmallows, plain
cc N/10, KOH
80
60
40
20
1. hour
2
3.

FIG. 26

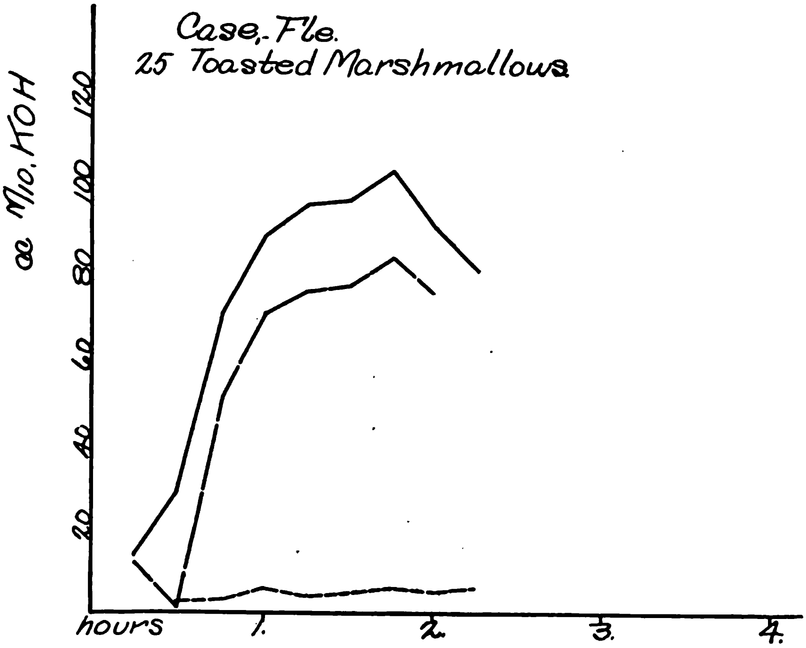
Case,-Fle.
25 Toasted Marshmallows
cc N/10. KOH
120
100
80
60
40
20
hours
1.
2.
3.
4.

FIG. 27

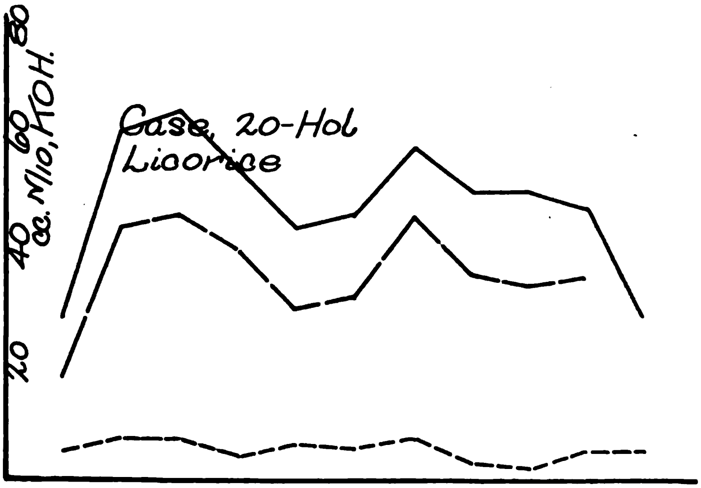

FIG. 28

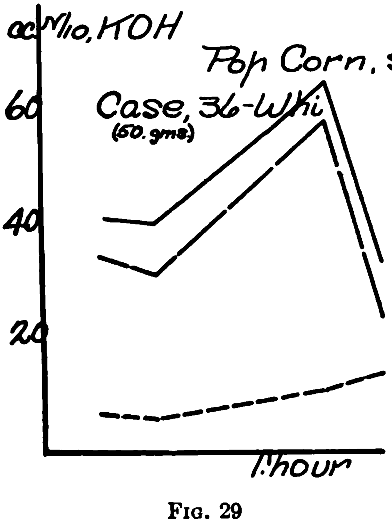

FIG. 29

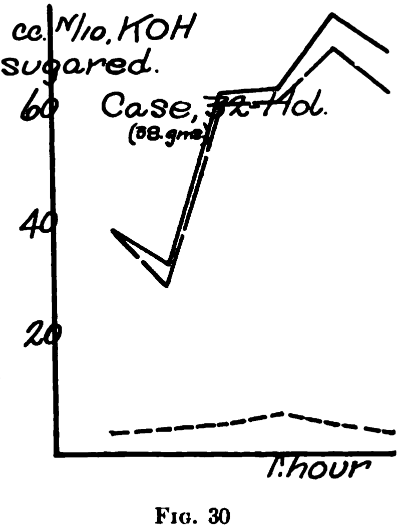

FIG. 30

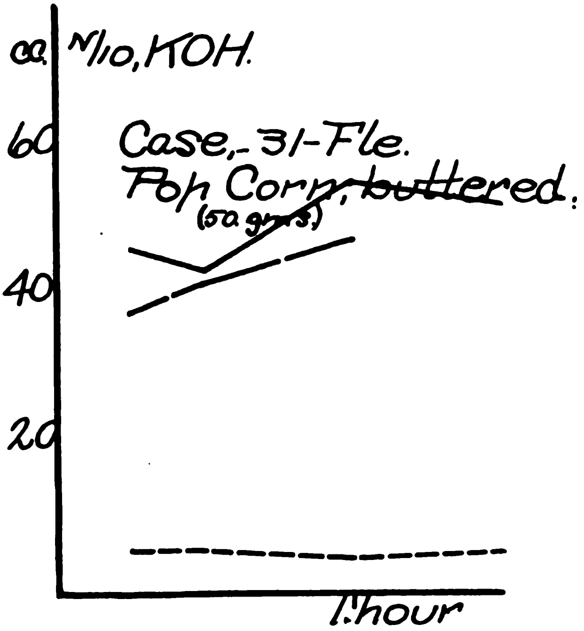
cc. N/10, KOH.
60
40
20
Case, - 31 - Fle.
Pop Corn, buttered.
(50 grms.)
1. hour

FIG. 31

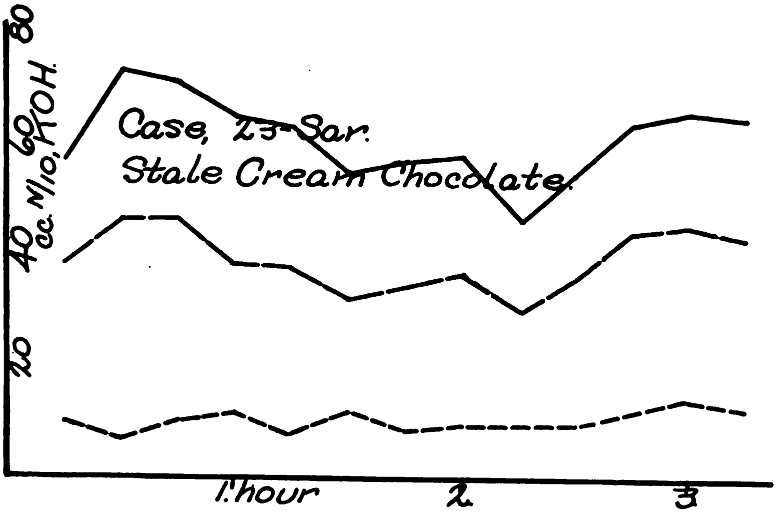
80
60
40
20
cc. N/10. KOH.
Case, 23 - Sar.
Stale Cream Chocolate.
1. hour
2.
3

FIG. 32

Marshmallows and licorice. Toasted and untoasted marshmallows were given to one of our subjects. The gastric response was markedly different in the two cases. The toasted marshmallows left the stomach an hour sooner and gave rise to an acid development forty points higher than plain marshmallows. The psychic stimulation due to the more appetizing flavor after toasting may be partly responsible for such differences, as well as the alteration in texture and in the condition of the egg white which they contain. It is known that raw egg white has very little stimulatory power as compared with the cooked product.

A man was allowed to suck a stick of licorice for 15 minutes, obtaining in this time about 5 grams of the substance. It gave rise to a fairly abundant secretion of moderate acidity and remained in the stomach for 2¾ hours.

Pop-corn preparations. Sugared pop-corn was given to two subjects in amounts of 50 and 38 grams respectively. Such pop-corn was found to leave the stomach in about 1 hour and to develop a moderate acidity. Buttered pop-corn gave a very similar response in another subject (see fig. 31), leaving for the most part in an hour and a half. It must be borne in mind, however, that certain of the larger and harder particles of corn which could not be aspirated remained in the stomach somewhat longer. Practically all of the corn left in moderate time.

Bread and honey. Sugars being very frequently given in the form of syrups added to bread and other foods, we endeavored to determine what effect such additions might have upon the gastric response.

A man was given 40 grams of whole wheat bread without additions, and after this had left the stomach he was given the same amount of bread with 20 grams of honey. The addition of honey depressed gastric secretion slightly but did not delay evacuation, although the food value of the preparation had been greatly increased. A moderate amount of honey added to bread cannot, therefore, be considered harmful.

TABLE 1

The response of the human stomach to candies

SUBJECT NUMBER	PREPARATION	EVACUATION TIME	HIGHEST TOTAL ACIDITY	TOTAL ACIDITY AT ONE HOUR
1. Rud	Dilute glucose solution	1:45	107.5	90.0
2. Ree	Dilute glucose solution	1:45	57.5	48.0
3. Rud	Concentrated glucose solution	2:15	106.0	28.0
4. Ree	Concentrated glucose solution	2:30	86.5	10.0
5. Rud	Dilute cane sugar solution	1:45	82.0	68.0
6. Ree	Dilute cane sugar solution	1:45	71.0	58.0
7. Ree	Concentrated cane sugar solution	2:45	99.5	95.0
8. Rud	Dilute maple sugar solution	1:15	88.5	86.0
9. Ree	Dilute maple sugar solution	0:45	32.5	32.5
10. Sea	Chocolate creams	2:15	56.5	56.0
11. Sar	Chocolate creams	3:15	79.5	54.0
12. Sar	Creams	2:45	36.0	24.0
13. Sea	Creams	2:30	57.5	44.0
14. Whi	Chocolate fudge	2:15	73.0	61.0
15. Sea	Milk chocolate	2:00	69.5	69.0
16. Sar	Bonbons	2:30	33.0	24.0
17. Whi	Cream wafers, peppermint	2:30	42.5	40.0
18. Whi	Cream wafers, strawberry	1:45	37.5	29.0
19. Whi	Lemon sticks	1:00	52.0	6.0
20. Hol	Lemon sticks	1:00	62.0	18.0
21. Sar	Caramels	2:45	80.0	71.0
22. Whi	Caramels	2:45	92.0	86.0
23. Sar	Taffy, salt water	1:45	26.5	26.0
24. Hol	Licorice	2:45	64.0	54.0
25. Ree	Gum drops	1:30	38.5	24.0
26. Fle	Marshmallows, plain	3:15	61.5	43.0
27. Fle	Marshmallows, toasted	2:15	103.0	88.0
28. Sea	Bread and honey	1:30	75.0	64.0
29. Whi	Pop-corn, sugared	1:30	65.5	58.0
30. Hol	Pop-corn, sugared	1:30	78.5	64.0
31. Fle	Pop-corn, buttered	1:30	56.5	56.0
32. Sar	Stale chocolate creams	3:15	72.5	64.0

SUMMARY AND CONCLUSIONS

Large amounts (100 grams) of cane sugar or glucose in concentrated solution markedly depressed gastric secretion and delayed evacuation of the stomach.

Small amounts (10 grams) of cane sugar or glucose did not appreciably inhibit either gastric secretion or evacuation.

Candies depress secretion and delay evacuation in proportion to their sugar content and the amounts of them ingested. This tendency is influenced, however, by flavoring substances, and particularly by added food ingredients such as milk, eggs or chocolate, which stimulate gastric secretion.

Candies should be eaten not before but after meals. Hard candies which must be sucked are preferable to cream candies for children because of the smaller quantity of less concentrated sugar solution derived from them.

Cane sugar and maple sugar elicited much the same response from the human stomach as glucose, although the possibility that the greater sweetness and less rapid absorption of the first mentioned sugars gives them a slight advantage is not excluded.

Soft candies such as bonbons, soft creamy wafers and the interiors of chocolate creams when given in 100-gram portions exerted the same depressing action on gastric secretion and evacuation as concentrated sugar solutions.

Peppermint oil used as a flavoring agent delayed evacuation while a strawberry fruit flavor appeared to accelerate it.

Chocolate appeared to stimulate gastric secretion as indicated by experiments on milk chocolate, chocolate fudge and chocolate creams, which gave higher acid figures than plain sugar candies. Stale chocolates remained in the stomach relatively long.

The sucking of hard candies introduced but a small amount of sugar into the stomach which was readily evacuated and exerted little depressing action on gastric secretion.

Chewing caramels gave rise to a more voluminous gastric secretion than cream candies, but evacuation times were about the same. Salt water taffy gave rise to less secretion, while gum drops left the stomach rapidly with little acid production.

Plain marshmallows remained in the stomach rather long, but after being toasted these confections left the stomach rapidly and gave rise to high intragastric acidities.

Licorice gave rise to a fairly abundant secretion and remained in the stomach for nearly 3 hours.

Sugared or buttered pop-corn developed a moderate acidity and left the stomach rather quickly.

The addition of honey to bread did not delay evacuation, although acid production was somewhat depressed.

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FURTHER EVIDENCE ON THE FUNCTIONAL CORRELATION OF THE HYPOPHYSIS AND THE THYROID

JOHN A. LARSON

From the Rudolph Spreckels Physiological Laboratory of the University of California

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In a former paper (1) I presented evidence to show that the administration of the anterior lobe of the pituitary has a very beneficial action upon maintenance and growth of thyroidectomized rats. In that paper I pointed out the importance of using animals of "approximately the same size, age and strain, and wherever possible from the same litter." At that time however, I was unable to meet these conditions in a wholly satisfactory way. I have now repeated the experiments with a larger number of animals and with strict regard to the comparison of individuals from a single litter.

There were in all seventy-two litters used. These were selected of as nearly the same date of birth as the conditions of the work would permit. The litters were kept separated and directly upon the day of weaning were ear-marked and subdivided into the respective series. In this arrangement extreme care was taken in regard to sex, weight and even color. All runts, individuals below the average weight and size of the litter, were eliminated. In anticipation of possible accidental deaths from ether, etc., the members in the operated series greatly outnumbered those in the normal series. There were four main series of animals:

A. Thyroidectomized animals fed upon the normal diet in addition to kidney.

B. Thyroidectomized animals fed upon the normal diet in addition to anterior lobe.

C. Normal, that is, unoperated, animals fed upon the normal diet in addition to anterior lobe.

D. Normal, that is, unoperated, animals fed upon the normal diet in addition to kidney.

To these series a fifth, E, was added, consisting of only a very few thyroidectomized animals which were fed upon the normal diet in addition to thyroid. As was to be expected from the results of thyroid feeding in cases of thyroid deficiency in other animals and also from the work of Cramer and McCall (2) on the rat, the animals in group E differed scarcely at all from the normals. The individual results will not be given in the tables, but the final results will be recapitulated with the other four series.

In the subdivision of the litter one rat of the same sex and color was placed in each of the four main series. This was possible in most cases, since the number in the litters varied from four to twelve. But if there happened to be, say, two males and two females in one litter, those of the same sex were placed in the operated series and the others in the unoperated. After picking out one animal for each series all of the remaining litter mates were distributed strictly according to sex in the operated groups, there being sometimes four from one litter in each operated series. Any odd member was placed in the fifth group. The result was that at the end of the division every member in each of the four series had a litter mate of the same sex in every other series. I have thus been able to give tables in which it is possible to see the results in each single litter although as a matter of fact the whole number of animals used was so large that a statistical treatment would have served the same purpose.

So far as possible, litter mates were operated upon on the same day.

The experiment lasted from August 1, 1919, the date of the first operation, until March 1, 1920, the time of the last weighing. The animals were weighed separately and at the same time, in relation to feeding, once a week until through December and then every two weeks until January 19, and the final weighings were made at the end of the experiment on March 1.

The rats were kept five in a cage. The cages contained only males or females in order to avoid distortion of growth curve through pregnancy. The food which I have spoken of above as "normal diet" consisted of the following ingredients:

Corn meal	6.0 parts
Rice	2.0 parts
Barley	2.0 parts
Meat powder	4.5 parts
Lard	5.5 parts
NaCl	1.0 per cent
$CaCl_2$	1.5 per cent
Greens ad libitum	

In addition to the normal diet each animal received as already stated either anterior lobe of the pituitary or an equivalent amount of kidney. The members of group E received 0.2 gram of fresh beef thyroid daily; whenever possible the dosage of anterior lobe was one entire lobe daily to each animal in the two series, and whenever there were too few glands for that dosage they were divided equally between the different members. The anterior lobes were administered in such a way that each animal received at least one-half a gland at each feeding.

The histories of all the members of the series are recorded in two main tables. Throughout the following discussion no reference is made to animals which were killed by ether or in a few cases, lost. Such individuals were discarded.

In the first table the histories of all of the animals still living at the termination of the experiment are exhibited apart from those rats which died. These latter are treated in tables 3 and 4.

Table 1 shows the sex, number, date of birth, date of operation, initial and terminal weights as well as the gain. Up to no. 65, the number, sex and date of birth are the same for all of the individuals represented in a horizontal row, since all of the animals therein are members of the same litter. In litters 65, 66 and 67, the members are of the same sex in the operated group but of a different sex from that in the normal groups, which is the same, however, in the two unoperated series. The members of groups 73 to 81 are from different litters owing to deaths of the other litter mates.

Table 2 presents the averages of the data shown in detail in table 1. The homogeneity at the outset of the experiment is indicated by the average initial weights which are 32.9, 32.5, 31.8 and 31.8 respectively. In contrast with these the average terminal weights (and the average gains) show marked differences. Growth was slower in the thyroidectomized animals fed on the control diet than in the thyroidectomized animals which received pituitary, the gain of the former being only 123.7 grams as compared with 171.3 grams. This result is in conformity with that contained in the previous paper. It must be remembered, moreover, that these figures deal only with the animals which survived at the close of the experiment. If in calculating the effect upon the rate of growth the animals which did not survive to the end had been included, the difference would have been still more marked.

It is also of interest to note that in the normal series the gain in weight was greater in the pituitary animals than in those receiving the control diet. Under the circumstances there can scarcely be a

TABLE 1

NUMBER OF LITTER	SEX	SERIES A					SERIES B					SERIES C			SERIES D			SERIES E		
		Date of birth	Date of operation	Initial weight	Terminal weight	Gain	Date of birth	Date of operation	Initial weight	Terminal weight	Gain	Initial weight	Terminal weight	Gain	Initial weight	Terminal weight	Gain	Initial weight	Terminal weight	Gain
				grams	grams	grams		grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
1	♂	6/22	8/14	38	204	166		8/13	39	238	199	36	262	226	37	206	169	35	218	183
2	♂	6/23	8/7	59	182	123		8/4	51	268	217	45	296	251	54	242	188	32	192	160
3	♀	6/24	8/12	21	136	115		8/12	27	195	168	25	174	149	26	182	156			
4	♂	6/24	8/13	26	100	74		8/12	25	190	165	30	230	200	28	224	196			
5	♀	6/24	8/14	41	130	89		8/13	35	176	141	25	186	161	26	174	148			
6	♀	6/25	8/11	41	174	133		8/11	37	170	133	36	186	150	35	150	115			
7	♀	6/25	8/15	26	126	100		8/15	38	198	160	31	230	199	25	202	177			
8	♀	6/26	8/11	39	126	87		8/11	35	195	160	36	180	144	33	170	137	24	144	120
9	♀	6/26	8/11	43	98	55		8/11	46	160	114	50	210	160	26	182	156	42	162	120
10	♀	6/26	8/12	28	166	138		8/12	34	240	206	33	170	137	35	150	115			
11	♀	6/26	8/14	33	144	111		8/13	29	190	161	33	204	171	28	150	122			
12	♀	6/27	8/15	30	122	92		8/15	36	195	159	32	154	122	35	112	77			
13	♀	6/27	8/15	30	174	144		8/15	36	220	184	42	142	100	38	172	134			
14	♂	6/27	8/14	29	198	169		8/13	35	250	215	34	264	230	34	228	194			
15	♂	7/3	8/22	36	210	180		8/20	35	260	225	51	272	221	47	204	157	47	248	201
16	♂	7/8	8/26	35	216	171		8/22	32	235	203	37	214	177	41	194	153	34	234	200
17	♂	7/9	8/27	38	192	154		8/27	26	208	182	34	302	268	35	258	223			
18	♀	7/9	8/29	35	164	129		8/24	33	160	127	36	204	168	25	168	143			
19	♀	7/10	8/19	33	112	79		8/18	39	168	129	26	136	110	23	144	121			
20	♀	7/10	8/22	41	186	145		8/20	30	232	202	28	194	166	24	176	152			
21	♀	7/10	8/22	28	174	146		8/20	30	196	166	32	168	136	30	124	99			
22	♂	7/10	8/22	29	174	145		8/21	39	266	227	33	264	231	37	220	183			
23	♀	7/12	8/14	32	180	148		8/14	31	232	201	35	130	95	36	170	134			
24	♀	7/13	8/26	36	178	142		8/29	33	208	175	27	180	153	27	180	153			
25	♂	7/14	9/1	22	304	282		8/29	30	202	272	20	304	284	21	308	287			

26	♀	7/26	9/4	29	136	107		9/3	26	206	180	33	174	141	35	156	121	27	180	153
27	♀	7/26	9/5	35	156	121		9/5	35	180	145	43	184	141	44	172	128			
28	♀	7/26	9/6	41	166	125		9/5	35	195	160	47	210	163	48	196	148			
29	♂	8/8	9/24	33	188	155		9/24	29	235	206	21	234	213	22	206	184	46	226	180
30	♀	8/8	9/26	22	134	112		9/26	18	195	177	28	176	148	27	190	163	32	178	146
31	♀	8/8	9/26	25	134	109		9/26	24	195	171	26	154	128	26	172	136			
32	♀	8/10	10/12	27	150	123		10/12	26	230	204	29	160	131	31	138	107			
33	♀	8/10	10/12	25	114	89		10/12	24	175	151	30	150	120	31	144	113	30	138	108
34	♀	8/11	10/6	26	138	112		10/6	35	170	135	28	166	138	28	164	136			
35	♀	8/11	10/2	34	136	102		10/2	33	190	157	19	160	141	19	134	115	31	154	123
36	♂	8/11	9/22	35	214	179		9/22	38	230	192	33	286	253	33	236	203	31	246	215
37	♀	8/12	9/25	34	164	130		9/24	21	230	209	31	188	157	33	178	145			
38	♀	8/12	10/15	34	132	98		10/7	34	160	156	31	180	149	34	196	162			
39	♀	8/13	10/15	33	152	119		10/7	31	170	139	32	170	138	32	164	132			
40	♂	8/13	9/25	33	236	203		9/24	30	204	174	27	250	223	27	250	223			
41	♀	8/13	10/22	30	140	110		10/23	30	224	194	31	188	157	29	182	153			
42	♂	8/13	9/22	52	174	122		9/22	46	170	124	30	232	202	28	170	142			
43	♀	8/13	10/20	22	162	140		10/20	40	192	152	28	204	176	34	166	132			
44	♀	8/13	10/16	44	140	96		10/20	27	195	168	30	210	180	33	158	125			
45	♂	8/14	9/23	38	214	176		9/23	24	250	226	35	242	207	35	204	169			
46	♂	8/14	10/19	26	136	110		10/23	27	210	183	31	240	209	29	226	197			
47	♂	8/14	9/22	39	84	45		9/22	26	240	214	22	236	214	21	180	159			
48	♀	8/14	10/15	30	160	130		10/9	30	175	145	37	180	143	34	152	118			
49	♀	8/14	10/6	26	80	54		10/7	28	180	152	32	172	140	34	122	88			
50	♂	8/16	9/25	28	162	134		9/24	24	240	216	50	292	242	35	216	181			
51	♀	8/18	9/24	21	108	87		9/26	20	195	175	41	200	159	44	232	188			
52	♂	8/18	9/30	21	170	149		10/1	29	246	217	21	222	201	22	232	210			
53	♀	8/19	10/15	24	138	114		10/15	24	190	166	37	132	95	39	130	91			
54	♂	8/19	10/23	26	164	188		10/22	37	185	148	41	256	215	35	244	209			
55	♂	6/28	8/14	45	148	103		8/16	32	235	203	36	239	203	38	206	188			
56	♂	6/27	8/14	43	214	171		8/16	72	235	163	34	212	178	34	228	194			
57	♂	6/22	8/1	35	100	65		8/15	34	130	96	23	278	255	28	270	242			

TABLE 1—*Concluded*

NUMBER OF LITTER	SEX	SERIES A					SERIES B					SERIES C			SERIES D			SERIES E		
		Date of birth	Date of operation	Initial weight	Terminal weight	Gain	Date of birth	Date of operation	Initial weight	Terminal weight	Gain	Initial weight	Terminal weight	Gain	Initial weight	Terminal weight	Gain	Initial weight	Terminal weight	Gain
				grams	*grams*	*grams*		*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
58	♂	6/22	8/7	59	182	123		8/13	25	230	205	31	280	249	31	216	185			
59	♂	7/11	9/1	23	54	31		8/29	26	220	194	35	288	253	35	242	201			
60	♂	8/8	9/24	22	120	98		9/24	24	240	216	36	230	194	34	230	196			
61	♂	8/16	9/30	34	162	128		9/22	38	220	192	43	286	243	42	240	198			
62	♂	7/15	8/27	32	202	170		9/5	45	260	215	33	286	253	39	260	221			
63	♀	8/19	10/23	32	148	116		10/22	31	180	149	23	200	177	28	206	178			
64	♀	8/19	10/26	28	140	112		10/26	28	230	202	35	278	243	36	228	192			
65	♀	7/11	9/1	31	182	151		9/23	33	180	149	♂29	204	175	30	226	196			
66	♀	7/15	9/1	36	164	128		9/22	21	149	128	♂26	204	178	25	242	217			
67	♀	8/28	9/25	28	184	156		9/7	31	230	199	♂38	196	158	35	258	223			
68	♀	7/17	9/16	47	150	103		9/16	31	180	149									
69	♀	7/13	8/29	27	170	143		8/29	29	160	131									
70	♀	6/25	8/14	32	152	120		8/13	34	168	134									
71	♀	6/23	8/7	59	160	101		8/14	25	200	175									
72	♀	6/24	8/13	23	192	169	6/26	8/19	33	160	127									
73	♀	7/23	9/3	40	146	106	6/26	9/5	25	96	71									
74	♂	6/24	8/15	27	154	127	6/23	8/12	31	130	99									
75	♀	8/19	10/26	27	134	107	8/16	8/13	42	146	104									
76	♀	7/14	8/13	34	146	112	7/12	8/29	49	244	195									
77	♂	6/23	8/14	47	90	43	6/26	8/12	38	100	62									
78	♂	6/24	8/15	32	152	120	6/26	8/13	25	176	149									
79	♀	7/11	9/1	30	100	70	7/15	8/29	27	182	155									
80	♀	6/24	8/11	31	158	127	6/26	8/5	37	260	223									
81							6/24	8/6	56	280	224									
Averages......................				32.9	156.6	123.7			32.5	203.8	171.3	31.8	212.7	180.9	31.8	193.5	161.7			

TABLE 2

Summary of results shown in table 1

	NUMBER IN GROUP	AVERAGE INITIAL WEIGHT	AVERAGE TERMINAL WEIGHT	AVERAGE GAIN
		grams	*grams*	*grams*
Series A............	80	32.9	156.6	123.7
Series B............	81	32.5	203.8	171.3
Series C............	67	31.8	212.7	180.9
Series D..........	67	31.8	193.5	161.7
Series E............	12	33.7	193.3	159.6

TABLE 3A

Average gain for each sex

	A. THYROIDECTOMIZED + KIDNEY			B. THYROIDECTOMIZED + ANTERIOR LOBE			C. NORMAL + ANTERIOR LOBE			D. NORMAL + KIDNEY		
	Average initial weight	Average terminal weight	Average gain	Average initial weight	Average terminal weight	Average gain	Average initial weight	Average terminal weight	Average gain	Average initial weight	Average terminal weight	Average gain
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Males.......	34.4	173.8	139.4	34	206.8	172.8	33.3	252.7	219.4	33.1	225.9	192.8
Females....	31.3	158.6	127.3	32	184.1	152.1	32.4	182.3	149.9	31.3	165.4	144.1

TABLE 3B

Total percentage gains; comparisons are made between members of the same sex under different conditions

	A. THYROID + KIDNEY	B. THYROID + ANTERIOR LOBE	DIFFERENCE BETWEEN A AND B	C. NORMAL + ANTERIOR LOBE	D. NORMAL + KIDNEY	DIFFERENCE BETWEEN C AND D
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Males........	405.2	508.2	103.0	658.8	582.4	76.4
Females......	306.2	406.7	100.0	462.6	425.7	33.9

TABLE 3C

Percentage gains of males over females; comparisons are made between the males and females receiving the same treatment. The figures here recorded represent the gains of the males over the females

A. THYROID + KIDNEY	B. THYROID + ANTERIOR LOBE	C. NORMAL + ANTERIOR LOBE	D. NORMAL + KIDNEY
per cent	*per cent*	*per cent*	*per cent*
9.5	13.6	46.3	36.3

TABLE 4

Animals dying during experiment, showing days of life after operation

NUMBER OF LITTER	SEX	DAYS OF LIFE	NUMBER OF LITTER	SEX	DAYS OF LIFE
Series A					
1	♂	204	36	♂	61
2	♂	83	38	♀	27
3	♀	201	42	♂	117
4	♂	152	43	♀	118
5	♀	189	44	♀	47
6	♀	37	45	♂	31
7	♀	44	48	♀	169
8	♀	46	49	♂	78
9	♀	24	51	♀	55
10	♀	168	52	♂	201
11	♀	192	53	♀	32
12	♀	133	54	♂	198
13	♀	24	55	♂	198
14	♂	24	56	♂	1
15	♂	196	57	♂	31
16	♂	38	58	♂	55
17	♂	44	59	♂	171
18	♀	23	60	♂	2
19	♀	76	61	♂	34
20	♀	131	62	♂	1
21	♀	23	63	♀	3
22	♂	166	65	♀	191
23	♀	195	66	♀	4
24	♀	168	67	♀	22
25	♂	127	70	♀	8
26	♀	24	71	♀	13
27	♀	31	72	♀	217
28	♀	182	4A	♂	93
31	♀	61	5A	♀	162
34	♀	71	56 A	♂	93
Series B					
3	♀	38	29	♂	31
10	♀	8	31	♀	34
15	♂	65	34	♀	32
21	♀	27	36	♂	41
22	♂	11	39	♀	127

doubt that the difference is due to a stimulating influence of the anterior lobe.

It is conceivable that the results might be different for the two sexes. I have tabulated the gains for the males and females separately in table 3. Here again the anterior lobe exerts a beneficial action upon the operated animals. In addition a comparison of the two sexes of the normal animal seems to reveal an increased effect of the anterior lobe upon the growth of males.

As stated above, tables 1 and 2 include only animals which were alive at the end of the experiment and are therefore useful in showing the influence of the gland substance on the rate of growth. Another important feature of the effect of the gland substance is seen in its influence upon the duration of life. Table 4 gives the length of life after the operation up to death and table 5 shows the total number of animals in each series as well as the number which died and the percentage of deaths.

TABLE 5

Tabular summary of deaths

	IN SERIES A	IN SERIES B	IN SERIES C	IN SERIES D	IN SERIES E
Total number	140	91	67	67	12
Number deaths	60	10	00	00	00
Number alive	80	81	67	67	12
Per cent deaths	42.8	10.9	0	0	0

DISCUSSION OF RESULTS

The experimental results show definitely that the administration of the anterior lobe of the hypophysis increases the growth and prolongs the life of thyroidectomized rats. Also the pituitary substance accelerates the growth of normal animals. These results at once suggest two possible interpretations: either *a*, that this beneficial effect is due to the direct substitution of the pituitary substance for that of the thyroid; or *b*, to a favorable action of the hypophysis upon the organism as a whole. The latter alternative is supported by the increased growth of the normal animals to which pituitary had been administered. On the other hand the difference in the two groups of operated animals is so much greater than in the two groups of normal animals that it hardly seems possible that this variance can be accounted for by a general improvement of the state of the animals but rather that it is due in part, at least, to an actual substitution of the hypophyseal substance for the lacking thyroid secretion. This possibility is rendered more probable by the effect of the pituitary on the prolongation of life of the operated animals. For a further discussion of these possibilities and the literature, reference is made to the previous paper (1).

Since the publication of the previous paper three investigations have appeared which have an interesting bearing. All three deal with the effect of the administration of pituitary to thyroidectomized frog larvae. Smith and Allen (4) working independently report negative results. Hoskins and Hoskins (3) on the other hand, not only obtained beneficial effects but found that pituitary causes a hastened metamorphosis. They interpret these results as indicating either a substitution or an effect of the pituitary upon the whole organism. Thus the results in the previous work, the first positive indication of the possibility of a substitution, have been confirmed by the author and by Hoskins on different animals.

The question now arises as to the cause of death of the operated animals. Post mortems were made of all the animals and in not a single case was there any macroscopic evidence of infection in the operated region. Invariably the animal was extremely emaciated, undersized and with scanty hair. The cause of death may be fairly attributed to metabolic disturbances and interference with the normal bodily functions due to lack of thyroid secretion.

Careful pathological examinations of three animals were made by three different pathologists. In one, no pathological condition save that of extreme emaciation could be detected, while in another evidences of a pneumonia were present. In the last one examined but little of significance was found, the pathologist agreeing with me that even if the animal had died of some terminal disease such as pneumonia the "evidence is sufficient to make a diagnosis of terminal pneumonia developing in an animal whose resistance had been lowered by abnormal disturbances of metabolism." Many of the animals which later died had remained for months in an emaciated, dwarfed condition. This condition did not develop till some time after the operation. As stated above, all so-called runts were excluded at the beginning. The retarded development then must have been due to thyroid deficiency.

Even if the deaths had been due to infection of any kind it would still be necessary to show why these infections attacked only the operated animal. Such a result would still be attributable to disturbed metabolism or lowered resistance. Again the scattered distribution of deaths would contra-indicate the incidence of an infection.

That the deaths are primarily due to a thyroid rather than a parathyroid deficiency might be inferred from the longevity of many of the animals after operation. As in the previous research in the cases of very young operated animals which died two to four days after operation some evidences of tetany were observed, only three cases in the present experiment. However the general condition of the animals up to death indicates a thyroid deficiency. Many of the operated animals exhibited what might be termed myxedematous symptoms. In addition to a dull lethargic condition there was a marked difference in the state of the coats of the operated animals and the normals. This condition in the operated individuals varied from dirty yellow coats to lack of hair in many spots. Three very unusual conditions were noted which the writer has not seen mentioned elsewhere. The most striking was in some individuals a complete loss of hair along the midline of the back. This gave the white rat a weird appearance, due to the bright red skin along the back in contrast to the white coat elsewhere. The bare place ran along the back and top of the head, being widest at the head. This condition was detected in about twelve of the operated animals. It appeared about the second week after operation and disappeared within four weeks, new hair growing and the general condition of the animal improving. This subsequent reappearance of hair is in accordance with the findings of Cramer and McCall (2) who state that after thyroidectomy in rats there is at first a fall in metabolism which is later followed by a return to normal or nearly normal condition. In eight other operated animals there was a peculiar dirty, pinkish coloration on the top of the head forming a V-shaped pattern. This appeared about four months after the operation and upon animals in poor condition, and persisted until death. In other animals, chiefly in those operated animals which received kidney, the hair disappeared completely in large irregular patches about the body. As time went on this condition grew worse. Seven of the operated rats became blind in one eye and two blind in both eyes. Of these one was in the pituitary group and the others in those receiving normal diet. Blindness has frequently been described in connection with pituitary disorders.

SUMMARY

The results of the present series of experiments show:

1. That the effect of administration of anterior lobe of the pituitary to thyroidectomized rats tends to prolong life and to accelerate growth. This confirms the results stated in the previous paper.
2. That pituitary feeding also noticeably increases the rate of growth in normal rats.
3. Anterior lobe seems to exert more influence upon the growth of the normal males than that of the normal females.

The writer wishes to express his gratitude and appreciation of Prof. S. S. Maxwell's interest and advice during the entire research.

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THE INFLUENCE OF AN ALCOHOLIC EXTRACT OF THE THYROID GLAND UPON POLYNEURITIC PIGEONS AND THE METAMORPHOSIS OF TADPOLES[1]

EMILY C. SEAMAN

From the Department of Experimental Medicine, Cornell University Medical College

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It has been found by Eddy (1) and other workers (2) that water-soluble vitamines can be extracted from various animal tissues. A study was made in this laboratory to ascertain whether the thyroid gland contains these substances and what physiological action, if any, they possess.

I. EFFECT OF EXTRACT ON POLYNEURITIC PIGEONS

Fresh glands were ground in a meat chopper and extracted with 95 per cent alcohol made 0.8 per cent acid with HCl. To 1000 grams of the chopped glands were added 2 liters of the acid alcohol. The jars containing the material were shaken in a shaking machine for two or three hours, and allowed to stand forty-eight to seventy-two hours. The extract was filtered, the bulk of the alcohol distilled off and the residue evaporated to dryness before an electric fan. The dry residue was dissolved in hot water, filtered, evaporated to dryness a second time and re-dissolved in 500 cc. of hot water and filtered. This filtrate was a dark brown solution, yielding a positive reaction with Biuret and Millon reagent, and a strong deep blue color with the special phosphotungstic acid reagent, the color reaching its maximum density after standing from two to three hours. One cubic centimeter of the extract contained 0.05 mg. of iodine and 0.435 per cent of nitrogen. Later attempts to isolate the vitamine fraction failed to separate it from the one containing iodine. The vitamine quality of the extract was studied by the curative effects on polyneuritic pigeons and, as a control on the effect of the iodine content, the "residue"[2] as prepared in the laboratory was used in corresponding doses. In every case the residue failed to produce any curative effect on the polyneuritic pigeons, demonstrating that it was not the iodine content which produced results. The extract showed rapid and marked curative properties in every case in which the whole extract was used. Separate extracts from different lots of glands were made. It was found when the same proportions of glands and acid alcohol were used that the final solution was not always of the same strength. This strength was standardized according to the iodine content and the final solution either evaporated or diluted until 1 cc. contained 0.05 mgm. of iodine. There was a slight variation in the nitrogen content.

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[1] From the Johnston Livingston Fund for Experimental Therapeutics.

The extract was divided into four portions:

a. The extract used as a whole.

b. Precipitation by phosphotungstic acid to isolate vitamine fraction after the method of Funk.

c. Extract treated with Lloyd's reagent and dry powder used.

d. Portion reserved for experiments on metamorphosis of tadpoles.

Results

The whole extract when used on polyneuritic pigeons gave marked and rapid curative results. The extract which had been treated with phosphotungstic acid, barium sulphate, mercuric chloride and H_2S, as outlined by Funk and Eddy (1), gave no results in any case. The administration of the powder, obtained after shaking the extract with

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[2] The thyroid "residue," as described in a previous communication from this laboratory, is the non-coagulable portion of a slightly alkaline extract of the glands after the nucleo-proteins have been removed. The hashed fresh glands are extracted for twenty-four hours at room temperature in tap water made faintly alkaline with NaOH. After straining through cheese-cloth, the filtrate is treated with 10 per cent acetic acid to precipitate the nucleoproteins. The acid filtrate is heated to boiling to remove the acid heat-coagulables; the filtrate from this made alkaline and again brought to boiling and filtered. This filtrate is neutralized and concentrated until 1 cc. of the filtrate contains 0.05 mg. of iodine. This thyroid "residue" produces, when injected into dogs, quite definite physiological reactions. In the stomach and pancreas it appears to stimulate the functions believed to be performed by the terminals of the gastric and pancreatic fibers of the vagus nerve. A neutral alcohol extract and the acidulated alcohol produce closely similar physiological effects, but clinically the acid alcohol extract does not seem as useful as either the neutral alcohol extract or the "residue."

Lloyd's reagent, gave positive results but the improvement was slower. The protocol for the administration of the whole extract given to polyneuritic pigeons is as follows:

Six pigeons were fed upon polished rice for weeks until extreme symptoms of polyneuritis occurred. Two pigeons were fed on normal pigeon food and used as controls. Two of the polyneuritic pigeons were given the "residue" by mouth in amounts corresponding to the iodine content of 4 cc. of the extract. In both cases no curative effect was produced in twenty-four hours after three doses of the residue. One pigeon who received only the residue died; the second, who received the residue and later the extract, survived.

With the four pigeons which were given the extract marked improvement was seen within six hours; demonstrating quite conclusively that the extract possessed curative properties for polyneuritic pigeons which the residue at this time did not possess.

Pigeon I. (*Vitamine extract*). Bird prostrate in cage and apparently dying. Weight dropped from 325 grams to 296 grams.

February 23, 10:00 a.m. 4 cc. extract by mouth
2:00 p.m. Feathers smoother, bird moving head, and eyes brighter; 2 cc. of extract by mouth
5:00 p.m. Bird crouched on legs, still unable to stand; 2 cc. extract given by mouth
February 24, 9:00 a.m. Walked strongly about cage; 4 cc. extract given by mouth
2:00 p.m. Bird apparently normal; flies across cage. When placed on floor of room, able to walk but totters a little after a dozen steps; 4 cc. extract given by mouth
February 25, 9:00 a.m. Bird walked strongly about floor of room. Dosage discontinued

Pigeon II. (*Vitamine extract*)

February 25. Weight dropped from 300 grams to 232 grams. Marked symptoms of paralysis.
11.00 a.m. 3.5 cc. of extract
4:50 p.m. Bird raising itself on legs if touched; 2 cc. extract given by mouth
February 26, 10:00 a.m. Marked improvement; bird perched on food pan, feathers smooth and bird apparently normal. No further dosage
March 15. Bird in good condition, flies and walks strongly. Put back on normal food

Pigeon III. (*Vitamine extract*)

February 25. Weight dropped from 257 grams to 221 grams. General paralysis.
11:00 a.m. 2 cc. extract given by mouth
4:30 p.m. 4 cc. extract given by mouth

February 26, 10:00 a.m. Marked improvement; 2 cc. extract given by mouth
3:00 p.m. Bird normal; 2 cc. extract given by mouth
February 27. Bird in such good condition, dosage discontinued

Pigeon IV. (Vitamine extract)

February 27. Weight dropped from 295 grams to 213 grams. Total paralysis.
9:00 a.m. 4 cc. extract given by mouth
2:00 p.m. Bird crouched on legs, unable to stand; 2 cc. extract given by mouth
February 28, 9:00 a.m. Bird walking about cage; 2 cc. extract given by mouth
March 1. Bird walks strongly with head erect
9:00 a.m. 2 cc. extract given by mouth
2:00 p.m. Bird normal; dosage discontinued

Pigeon V. (Residue)

February 26. Weight dropped from 312 grams to 241 grams. Extreme symptoms of polyneuritis.
10:00 a.m. 4 cc. residue given
February 27, 9:00 a.m. Bird dead

Pigeon VI. (Residue and extract)

March 1. Weight dropped from 297 grams to 231 grams. Extreme symptoms of polyneuritis.
9:00 a.m. 4 cc. residue given by mouth
2:00 p.m. No results; 2 cc. residue by mouth
5:00 p.m. No improvement; 2 cc. residue by mouth; 4 cc. extract given by mouth
March 2. 9:00 a.m. Bird living and crouched on legs; 2 cc. extract given
11:00 a.m. 2 cc. extract given
2:00 p.m. Bird improved but totters about cage
5:00 p.m. 2 cc. extract given
March 3. 9:00 a.m. Bird tottering about cage; decidedly improved but not normal; 4 cc. extract given
2:00 p.m. Bird walking normally; 2 cc. extract used.

Note. This bird never regained its normal vigor. It continued to improve and walked and flew about its cage but much of the time remained crouched down. After ten days it was put back on normal food, but even then showed less vitality than the other birds.

Conclusions

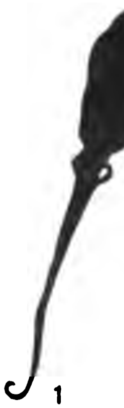
From the above experiment with polyneuritic pigeons it was demonstrated that the water extract from the thyroid glands made by 95 per cent alcohol made 0.8 per cent acid with HCl possessed the same curative property which has been attributed to water-soluble vitamines.

II. EFFECT OF ACID ALCOHOLIC THYROID EXTRACT ON METAMORPHOSIS OF TADPOLES

It has been found by Gudernatsch (3) and others that the metamorphosis of young tadpoles is hastened by feeding thyroid glands. Gudernatsch found that feeding the whole gland produced the hind legs in nine days after feeding, and the fore legs two days later. When these thyroid-fed tadpoles put out their anterior limbs and began to shorten their tails, they were eighteen to twenty days old. Swingle (4) obtained similar results by feeding iodoform, potassium iodide and iodine crystals. His most rapid results were obtained from the iodine crystals, limb buds appearing "in a few days." More tardy results were obtained with iodoform and potassium iodide.

It is claimed by Swingle and other workers that iodine is the active principle which hastens the metamorphosis of tadpoles, and this theory is generally accepted. In the following experiments done in this laboratory with the acid alcoholic extract, controls were made with iodine crystals, potassium iodide, thyroid nucleoprotein and the thyroid "residue" in amounts with corresponding iodine content.

Rana pipiens, approximately 15 mm. in length, were used and kept in tap water which was changed every day. To the fresh water were added the various substances in doses corresponding to 0.10 mg. of iodine a day. Each jar contained fifteen or twenty tadpoles and the experiments were repeated four times. A total of not less than sixty tadpoles was used for each substance, but out of this number at least fifteen died before complete metamorphosis. By far the most rapid results were obtained with the thyroid extract prepared by extracting the glands with 95 per cent alcohol made 0.8 per cent acid with HCl, as described in part I, and which proved to have the property of water-soluble vitamines. The limb buds appeared within eight hours, and complete metamorphosis with the bulging eyes and disappearance of the tail within four days. By the time complete metamorphosis had occurred in these specimens, only slight signs of limb buds appeared in the specimens treated with the iodine crystals, and no change occurred in the specimens fed the other substances. Negative results were obtained with the nucleoprotein and "residue," after continuing the feeding nearly three weeks. As the "residue" and alcoholic extract contained the same amount of iodine (1 cc. containing 0.05 mg. iodine) and no results were obtained with the residue or nucleoproteins, it is evident that the iodine content alone is not the only factor. It is



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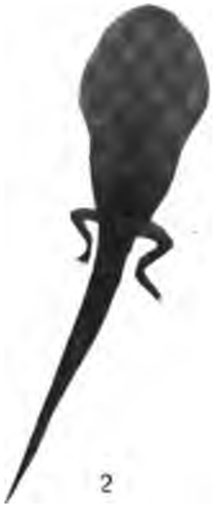

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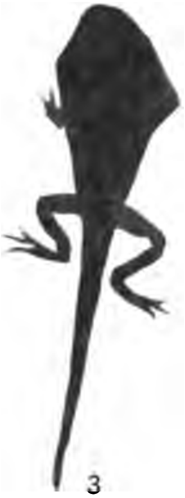

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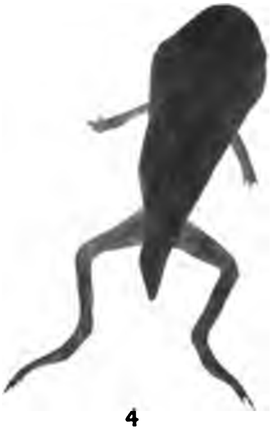

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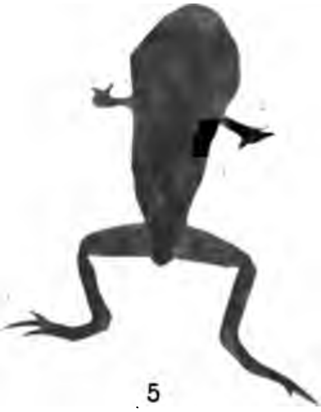

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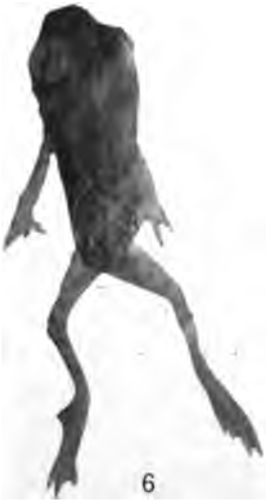

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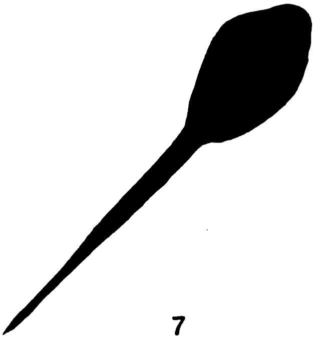

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Fig. 1. Less than twenty-four hours
Fig. 2. Thirty-six hours
Fig. 3. Forty-eight hours
Fig. 4. Three days
Fig. 5. Four days
Fig. 6. Five days
Fig. 7. Control

very probable that the iodine of the thyroid gland persists in more than one combination. The alcoholic extract may have contained, and according to Kendall (5) did contain, a substance not present in the residue, but Kendall did not obtain any such rapid results in the metamorphosis of tadpoles after feeding them "thyroxin" as took place with this alcoholic extract.

The following table represents the condition of the specimens when the experiment was performed on tadpoles approximately ten days after development from the egg. All the specimens did not develop in exact uniformity of time, there being a lag of four to six hours in some, but all showed complete metamorphosis by the end of the fourth day.

Table of results

HOURS	CONTROL	CRYSTALS	POTASSIUM IODIDE	NUCLEO-PROTEIN	RESIDUE	ACID ALCOHOLIC EXTRACT
12						Appearance of limb buds
36						Growth of legs
48						Emaciation begun. Growth stopped
72		No increase in growth				Tail beginning to disappear
96	Growth	Limb buds				Complete metamorphosis

See figures.

Effect of age on metamorphosis

The above experiment was repeated with four sets of tadpoles of the same age but hatched from eggs procured from different stores. The results were the same in each experiment.

When the experiment was repeated two weeks later, when the tadpoles were a month old, the development was slightly retarded, complete metamorphosis occurring not later than six days. In the experiment with tadpoles two months after they had been hatched, complete metamorphosis took place within ten days. Fewer specimens died when the older tadpoles were used.

GENERAL CONCLUSIONS

It has been found that the thyroid gland, as well as other tissues of the body, contains water-soluble vitamines, the property being demonstrated by the curative action on polyneuritic pigeons.

It was also found that the acid alcoholic extract had a marked accelerating action on the metamorphosis of tadpoles, the most rapid development occurring in specimens two weeks after they had been hatched, and slightly slower development as the age increased.

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THE ALKALI RESERVE IN EXPERIMENTAL SURGICAL SHOCK

BERNARD RAYMUND

From the Hull Physiological Laboratory, University of Chicago

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INTRODUCTION

The work herein reported was taken up in response to suggestions made by the Sub-Committee on Shock of the Committee on Physiology, National Research Council. Since the question of acidosis as a factor in shock became of such general interest, it seemed desirable to devote attention first to this, and leave for further consideration other factors, perhaps of equal importance. Hence this paper will be confined to the presentation of such data as have been accumulated in this laboratory with regard to the alkali reserve in shock.

Attention was first directed to the possible rôle of acidosis in the condition of shock by the claim of Spiro (1) in 1902 that the administration of hydrochloric acid to rabbits, or the intravenous injection of sodium hydrogen phosphate in dogs, was followed by "shock" effects. Howell (2) stated in 1903 that the intravenous injection of a 0.5 per cent sodium carbonate solution had a beneficial effect upon animals in shock but took the view (3) that this was due primarily to its action upon the heart. Apparently the question of its neutralizing effects did not enter into consideration. Dawson (4), using sodium bicarbonate after hemorrhage, reached the same conclusion. Seelig, Tierney and Rodenbaugh (5) using repeated small injections of sodium acid phosphate, stated that they were able to bring about a condition of rather profound shock in dogs, as judged by blood pressure readings. On the other hand, intravenous injections of sodium bicarbonate proved of decided benefit to animals in shock, but the authors judged that it acted directly upon the heart muscle. Yandell Henderson in 1910 (6) stated it to be his belief that the fatally rapid transudation of fluid from the veins in shock was due to the action of acidosis bodies upon the proteins of the tissues, causing them to imbibe water. He admitted that the process was probably very complex. Crile attempted to show in 1915 (7) by histological methods that the effects of the injection of sodium acid phosphate upon brain, liver and adrenal cells were in all respects similar to those found after death from surgical shock, and that such post-operative lesions could be prevented by the previous injection of sodium bicarbonate. He declared (8) that the bases of the body are in many cases exhausted by acid products developed during operations and recovery thereby rendered impossible. Corbett however (9) was unable to demonstrate any increase in urinary ammonia during traumatic shock, nor any increase in the hydrogen ion concentration of the blood, and concluded that the relation of acidosis to shock was not clear.

At the time this work was begun, Cannon (10) had stated in an informal memorandum to the Committee on Physiology of the National Research Council that "it is probable that the acidosis is causally related to the clinical condition presented in shock. . . Arterial pressure falls (vascular walls relax and cardiac contractions become less vigorous) when acid is injected experimentally into the blood vessels." Furthermore "a fairly close inverse relation exists between the degree of acidosis and the height of the arterial blood pressure—the more marked the acidosis the lower the pressure" and "as the degree of acidosis increases the general condition of the patient becomes worse—the greatest degree is found near death."

Early in the next year Cannon (11) reported work undertaken with Bayliss which seemed to show that "if the alkaline reserve is diminished by very slow injection of acid (N/2 HCl) into a vein, there is a fall of blood pressure to the shock level after the alkali reserve reaches a certain critical point (38 per cent in the cat). . . . Bruising or mashing the muscles of the hind legs is followed by a progressive fall in alkali reserve, and when the critical point is reached the blood pressure falls to the shock level." However in attempting to repeat the experiments with acid Bayliss (12) was unable to obtain the fall "except in cats obviously unhealthy."

Basing his assumptions upon this previous work Cannon (13) suggested that an excessive production after wounds of some acid metabolite and its distribution through the circulation might cause dilatation of the blood vessels, excessive lymph formation in all parts of the body and thus bring on exemia or shock. Similarly Henderson, Prince and Haggard (14) found that the carbon dioxide capacity of the whole blood could be greatly reduced by shock procedures and ventured the opinion that the acidosis of shock and of ether anesthesia is compensatory to, or a result of, the acapnia produced by hyperpnoea. In his first paper in 1918 Cannon (15) while still emphasizing the importance of acidosis in shock does not assign to it a causative rôle, merely stating that the drop in alkali reserve under anesthesia is greater the less the original margin of safety in the individual, and that such cases showed an ominous fall of blood pressure when operated, especially if at the time in a state bordering upon shock. In subsequent papers (16), (17) he stated that "as the blood pressure falls there is a loss of the alkali reserve of the blood (acidosis) roughly corresponding to the drop in pressure." In other words, acidosis had been relegated to a position of secondary importance, although Cannon still maintained that the injection of sodium bicarbonate was of prophylactic value in cases of shock.

Practically the same conclusion as to the importance of acidosis in shock had been reached independently by Guthrie (18) and McEllroy (19), although Henderson and Haggard (20), (21) stated that if in dogs under ether anesthesia "the level of CO_2 and alkali is reduced below the critical value lying between 33 and 36 volumes per cent of CO_2, a condition of general depression of all functions results. . . . If the marked resistance to further depletion of the carbon dioxide capacity, which occurs at the critical level, is broken down and the CO_2 capacity further reduced, the result is a condition of vital depression from which the subject does not spontaneously recover. This condition may be termed *acapnial shock.*" A low carbon dioxide capacity they regard as one of the definite criteria of shock. Subsequently Gesell (22) has shown that after either hemorrhage or visceral trauma, the reduction in the alkali reserve of the blood tends to be compensated for by a transfer of alkali from the tissues, and that the injection of N/2 HCl into normal animals is followed by indications of cardiac failure following a rise in arterial blood pressure. In this case also there is a passage of alkali from the tissues into the blood, so that successive doses of acid become less efficient in lowering the alkali reserve. Likewise Gasser and Erlanger (23) state that the alkali reserve does not show a sharp decline until after the animal is in a relatively advanced stage of shock, and that the reduced CO_2 capacity is the result, and not the cause of the fall in the arterial pressure.

CRITERIA OF SURGICAL SHOCK

Although the criteria for surgical shock are well established and quite generally recognized, most of the workers in the problem have shown a tendency to set some certain arterial blood pressure as the shock level, so-called, and to use this as a basis for determining at what time the animal passes into a state of shock, and to what degree. That the level of arterial pressure may be quite high when an animal is in true shock, and that the converse may just as readily be true, was pointed out by Meltzer (24) in 1908. This conception is given additional emphasis by Mann (25). He proposes as criteria of shock the following signs, which have become generally adopted: *a*, loss of sensibility; *b*, pallor of the mucous membranes; *c*, small, weak pulse; *d*, irregular, rapid, shallow or gasping respiration; and *e*, markedly lowered blood pressure (one-third to one-fourth of original level).

In view of the uncertainty as to what really constitutes a shock level of blood pressure, it seemed desirable on the whole to adopt these criteria as being the most satisfactory we possess, and in the experiments described in this paper they were constantly used in deciding whether or not the animal was in true shock. More emphasis was placed upon the first sign than upon the others, and in addition it was found that Guthrie's respiratory sign (26) often proved of value. This consists in a markedly prolonged inspiratory pause, and while not always present, is, when it appears, a reliable index, marking the beginning of the terminal respiratory paralysis.

EXPERIMENTAL PROCEDURE

Ether was used exclusively as the anesthetic, being given intratracheally and at such rate that the lid reflex was not abolished at any time during the experiment. In some experiments the patellar reflex was also used as an index of the depth of narcosis, although it was evident that it is neither as sensitive to the effects of ether nor, in the conscious dog, to those of prolonged low blood pressure, as the lid reflex. Thus in one experiment without ether and under local anesthesia, it was elicited after the corneal reflex had disappeared and when the heart was only beating with each occasional inspiration. That with the ordinary equipment it was possible in this way to keep the effects of the anesthetic at a minimum is shown in the protracted control experiments to be described later.

In order to obtain uniform results the dogs were not fed for eighteen hours previous to the experiment. Such exceptions as occur are noted. Determinations of the alkali reserve were made by Van Slyke's direct method (27). The blood was drawn either from the saphenous vein or, when the dogs were under ether anesthesia, from the femoral or carotid artery, oxalated, and immediately centrifuged for ten minutes, usually in a stoppered tube. Precautions to prevent the escape of carbon dioxide, such as the use of paraffin oil, were not taken. Peters (28) has stated that if the blood is centrifuged within fifteen minutes, the shifting of the carbonate into the erythrocytes does not occur, when the surface of the blood exposed to the air is small. In every instance the blood used in these determinations was centrifuged not more than ten minutes after being drawn. The plasma was saturated with alveolar air and analyzed in the usual manner. In a few cases it was allowed to stand over night in the refrigerator, but the analyses were checked by preserving a sample that had been analyzed beforehand and redetermining the alkali reserve the next morning. In no case could any change be detected. The results of the analyses, made in duplicate, were calculated to volumes per cent carbon dioxide at 0°, 760 mm. pressure, on the basis of saturation at 37°C., according to Van Slyke's formula. When parallel determinations were made on the whole blood the sample was divided into two portions, and while one was being centrifuged the other was saturated and analyzed. The reading so obtained was corrected for oxygen, etc., by the use of a few drops of 10 per cent sodium hydroxide solution.

Blood pressure and respiration were recorded in the usual manner. In most of the experiments shock was induced by a uniform technic, essentially that adopted by all workers in the field. After the blood pressure had reached a fairly stationary level, the abdomen was opened, the ether discontinued, the intestines removed and handled gently and continuously for fifteen minutes. Whereupon the guts were at once replaced and the abdomen closed securely with hemostats. From this point on the animal was kept warm, either by an electric light or by a heating pad, in order to eliminate the factor of lowered body temperature. If after a certain lapse of time, preferably an hour, the blood pressure showed no permanent lowering, another fifteen-minute period of trauma was resorted to as before. Occasionally the trauma had to be repeated several times before the animal showed any indication of shock; but except in one or two of the earlier experiments, the intestines were always replaced and not allowed to lie exposed to the air.

By closing the abdomen the degree of trauma can be much more accurately gauged, and there is furthermore no interference with the muscles of respiration.

In highly resistant animals there was sometimes resort to traction on the kidney, trauma to the liver or to the urinary bladder. These measures, especially the first, always caused a great disturbance in blood pressure. Such measures as clamping the inferior vena cava, occlusion of the portal system or of the abdominal aorta (29), (30), (31), seemed unnecessarily severe and were not used. Moderate trauma to the intestines in the manner described is sufficient to produce what appear to be the main clinical signs of shock, and the method can be controlled with fair accuracy.

In five of the experiments the procedure described above was followed, except that the entire gut was inflated with moist air at 40°C. to a pressure somewhat above the systolic blood pressure for a period of ten minutes. Five minutes were allowed for tying off the stomach and rectum, inserting the cannula in the caecum, and for deflating the gut and replacing it in the abdominal cavity. The guts were kept covered with hot towels throughout the entire period. This method is by far the most satisfactory of any that were tried, and makes it possible to control the degree of trauma with almost quantitative accuracy. By abolishing the circulation in the gut, asphyxia of the vessel walls and probably of the smooth muscle cells is set up, yet on the other hand the trauma is not so severe as to abolish the myenteric reflex. This is a very desirable feature, since it is doubtful if operative procedures in man ever completely do away with the reflex.

In seven experiments the method of Mann (32) was followed in inducing shock. All the structures in each limb with the exception of the supplying artery were tied off with iron wire ligatures. In this method the trauma is continuous throughout the experiment, but is possibly less severe than that involved in the foregoing procedures. Hence it is easier to follow the course of shock, and in this respect the method possesses certain advantages.

TYPES OF SHOCK

Naturally, if it is desired to compare the susceptibility of animals to trauma under various conditions, some certain basis for comparison must be found. By many workers in the problem the shock level of arterial blood pressure has been set at 50 mm. of mercury. But since this figure is admittedly arbitrary, and since its adoption is not universal, it seemed inadvisable to compare the resistance of animals to trauma on the basis of the length of time, after beginning shock procedures, that the blood pressure remained above this level. As the only alternative it was necessary to select as a criterion of susceptibility the *survival time* of the animal, that is, the length of time it remained alive under the conditions of the experiment, after beginning traumatization.

Using this as a basis it was found that the animals studied fell into four general classes, that is, there were in all four *types of shock*, so-called. These were as follows:

Type I. With one or two exceptions, in the dogs showing this type of shock, only one fifteen-minute period of trauma was needed to set up a serious condition. Of a total of thirty-one dogs, five, or 16.1 per cent, showed type I.

Maximum survival time	2 hours, 10 minutes
Minimum survival time	0 hour, 52 minutes
Mean	1 hour, 27 minutes

Type II. Dogs showing this type of shock had to be given two fifteen-minute periods of trauma, but not more. Ideally these two periods should have been an hour apart, but this rule could not be followed very closely. When shock was produced by Mann's method (see above) the trauma was of course continuous. However I have included two of the dogs so traumatized in this class, since the survival time of each was found to lie within the proper limits. Of a total of thirty-one dogs, eight, or 25.9 per cent, showed type II.

Maximum survival time	2 hours, 47 minutes
Minimum survival time	1 hour, 20 minutes
Mean	2 hours, 9 minutes

It will be seen that type I and type II overlap somewhat and that the distinction between the two is in a measure arbitrary. This however is not the case with the last two types, which are clearly set off from the first two and from each other.

Type III. In this type are included the dogs which required as a rule more than two periods of trauma to seriously alter their condition. Of a total of thirty-one dogs, thirteen, or 41.9 per cent, showed type III. This includes three dogs traumatized by ligating the four limbs. All of these dogs died in true shock except one (exper. 90) which died of accidental hemorrhage. However this dog showed the same rate of fall of blood pressure as the others in this class, previous to hemorrhage, and so it is included with them.

Maximum survival time	5 hours, 32 minutes
Minimum survival time	2 hours, 56 minutes
Mean	4 hours, 15 minutes

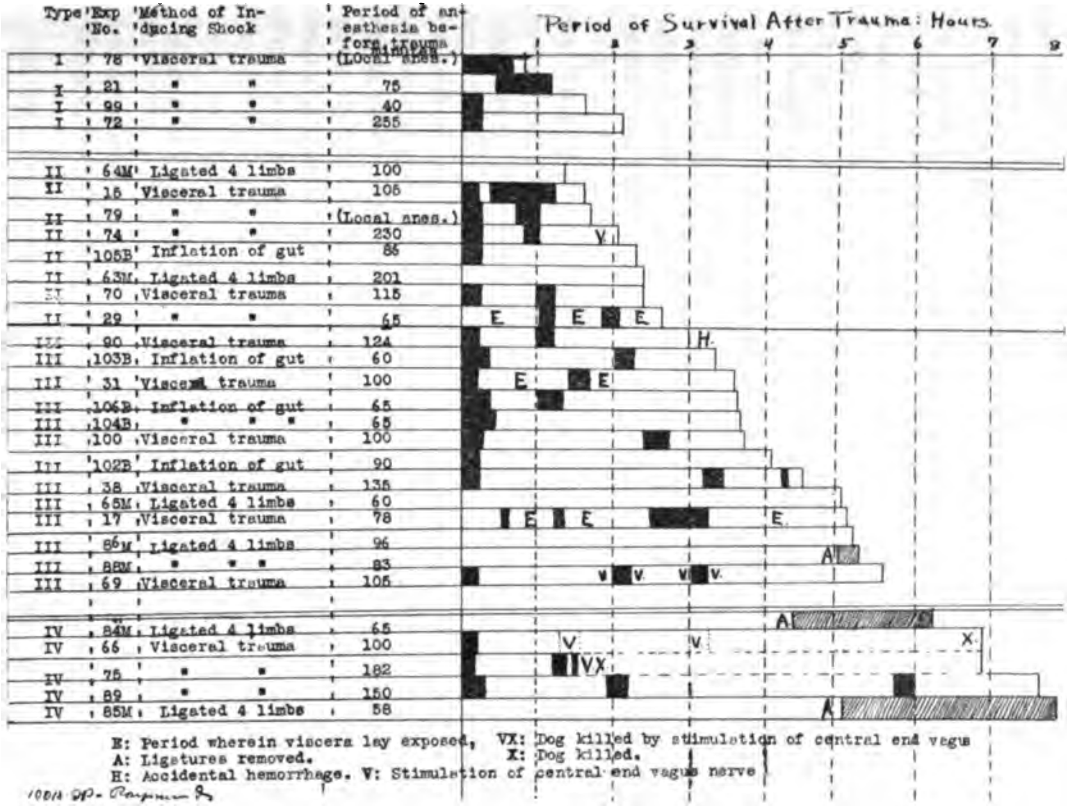
Type IV. Since the dogs in this class did not show typical shock, it is perhaps a misnomer to characterize this as one of the four types. However for the purpose of convenience this misnomer will be allowed to stand. Of a total of thirty-one dogs, five, or 16.1 per cent, showed type IV. This includes two dogs traumatized by Mann's method.

Maximum survival time	7 hours, 52 minutes
Minimum survival time	6 hours, 46 minutes
Mean	7 hours, 15 minutes

All of the dogs showing the first two types of shock gave a profound fall in blood pressure within thirty minutes after beginning the initial trauma. I had at first thought of using the degree of such fall as a criterion of susceptibility, and of determining the types of shock upon this basis. But the fall varied between such wide limits, 2 mm. to 102 mm., that this was impossible. Of the dogs exhibiting type III shock, all but three showed a fall in blood pressure (2 mm. to 49 mm.) thirty minutes after beginning initial trauma, while these three gave a rise in pressure (2 mm. to 36 mm.). However on classifying the data it was found that, without exception, every dog showing type IV shock gave a rise in pressure (3 mm. to 21 mm.) at the end of the thirty-minute period. This seems very significant, even granting that in type III shock such a rise of pressure may be seen. It shows that some notion of the course of shock may be obtained thus early in the experiment. This time rather than some other was chosen at which to make the observations on blood pressure, for the reason that at this time the wide fluctuations in blood pressure set up by the trauma have disappeared, while general and widespread damage to the circulation presumably has not had opportunity to occur.

The data summarized above are shown in graphical form in figure 1.

In the figure the heavily shaded areas represent periods wherein the intestines were traumatized. In the experiments in which the four limbs were tied off the block representing the survival time is left unshaded. In three such experiments where the ligatures were removed before the close of the experiment (indicated by the letter *A* in the



Type	Exp No.	Method of Inducing Shock	Period of anesthesia before trauma Minutes
I	78	Visceral trauma	(Local anes.)
I	21	" "	75
I	99	" "	40
I	72	" "	255
II	64M	Ligated 4 limbs	100
II	15	Visceral trauma	105
II	79	" "	(Local anes.)
II	74	" "	230
II	105B	Inflation of gut	85
II	63M	Ligated 4 limbs	201
II	70	Visceral trauma	115
II	29	" "	65
III	90	Visceral trauma	124
III	103B	Inflation of gut	60
III	31	Visceral trauma	100
III	106B	Inflation of gut	65
III	104B	" " "	65
III	100	Visceral trauma	100
III	102B	Inflation of gut	90
III	38	Visceral trauma	135
III	65M	Ligated 4 limbs	60
III	17	Visceral trauma	78
III	86M	Ligated 4 limbs	96
III	88M	" " "	83
III	69	Visceral trauma	105
IV	84M	Ligated 4 limbs	65
IV	66	Visceral trauma	100
IV	75	" "	182
IV	89	" "	150
IV	85M	Ligated 4 limbs	58

E: Period wherein viscera lay exposed, VX: Dog killed by stimulation of central end vagus
A: Ligatures removed. X: Dog killed.
H: Accidental hemorrhage. V: Stimulation of central end vagus nerve

Fig. 1. Showing survival time of dogs after trauma and types of shock.

figure) the period after removal is indicated by cross-shading. Further, in these experiments trauma was considered to have been begun when both hind-legs had been ligatured off and before the fore-legs had been ligatured. In three early experiments in which the guts were allowed to lie exposed, such periods of exposure are indicated in the figure by the letter *E*. Prolonged stimulation of the central end of the vagus is indicated by the letter *V*, either in conjunction with trauma to the viscera, as in experiment 69, or alone. In experiment 75 where the dog was killed by such stimulation while the arterial blood pressure was still 123 mm., the fact is indicated by the letters *VX*. The letter *X*, experiment 66, indicates that the dog was killed with ether in order to bring the experiment to a close. It will be seen in column 4 of the figure that the length of the preliminary etherization period varied from one hour or less to more than four hours and fifteen minutes in one experiment. However it is improbable that this had much effect upon the survival of the animal (see control experiments, table 1, below).

Five experiments were performed with Dr. A. C. Ivy under local anesthesia, without the use of ether, aseptic precautions being observed. Blood pressure and respiration were recorded in three of these experiments; in all the plasma alkalies, red cell count, hemoglobin and corpuscle volume were determined at intervals. Only one of the dogs was strictly normal. The others had had the vagi and splanchnics severed above the diaphragm and the coeliac ganglion extirpated under asepsis some two or three weeks previously. However these with one exception have been included in the analysis given above. Since the experiments were few in number it is not proposed here to draw any conclusions from them save as regards survival time.

THE ALKALI RESERVE IN NORMAL DOGS NOT SUBJECT TO ETHER

Sixty-eight determinations of the plasma alkalies were made on forty dogs not subjected to ether. The results may be summarized as follows:

Maximum alkali reserve............	59.5 volumes per cent (exper. 68)
Minimum alkali reserve............	32.4 per cent (expers. 4 and 20)
Mean..............................	43.4 per cent (68 determinations)
Mean heart rate....................	105
Mean respiration...................	31

The dog showing the maximum alkali reserve had been fed.

In fourteen dogs, eighteen determinations were made of the alkali reserve of the whole blood, as follows:

Maximum alkali reserve	55.6 per cent (exper. 101)
Minimum alkali reserve	36.6 per cent (exper. 92)
Mean, whole blood	47.0 volumes per cent
Mean heart rate	108
Mean respiration	23

None of these dogs had been fed. What effect feeding might have on the alkali reserve was not definitely determined, but from the data in hand there would seem to be no intimate relationship: that is, the alkali reserve did not invariably increase after feeding. Of course this would depend largely on the character of the food.

The figures given above agree with those found by Morriss (33) for plasma, but not with those cited by McEllroy (19). Henderson, Prince and Haggard (14) give 48 volumes per cent as the normal reserve of the whole blood in the dog.

THE ALKALI RESERVE IN DOGS UNDER CONTINUOUS ETHER ANESTHESIA

In determining the effect of the anesthetic upon the alkali reserve, arterial plasma was used, except in the first two experiments where the analysis was made upon the plasma of venous blood throughout. Although comparison is made here between the alkali reserve of the normal venous plasma and that of the arterial plasma after etherization, the error introduced thereby is probably slight and no doubt constant. The results are given in table 1.

For the sake of convenience the etherization may be divided into four periods of unequal length, viz.: period 1, first 45 minutes of ether anesthesia; period 2, 45 minutes to 2 hours; period 3, 2 to 4 hours; period 4, beyond 4 hours. On this basis the data presented in the following table may be summarized briefly as follows:

Period 1. First 45 minutes of ether

Number of determinations	18
Maximum alkali reserve	41.9 per cent (experiment 43)
Minimum alkali reserve	19.8 per cent (experiment 54)
Mean	33.9 volumes per cent
Maximum fall from normal	25.1 per cent (experiment 54)

Minimum fall	1.3 per cent (experiment 46)	
Mean fall	10.5 per cent	
Mean heart rate		189
Mean increase in same		91
Mean respiration		64
Mean increase in same		31

Period 2. Ether, 45 minutes to 2 hours

Number of determinations		18
Maximum alkali reserve	35.3 per cent (exper. 30)	
Minimum alkali reserve	16.4 per cent (see note 2, above)	
Mean	28.0 volumes per cent	
Maximum fall from normal	34.4 per cent (see note 3, above)	
Minimum fall from normal	4.0 per cent (exper. 30)	
Mean fall	15.8 volumes per cent	
Mean heart rate		151
Mean increase in same		45
Mean respiration		69
Mean increase in same		26

Period 3. Ether, 2 to 4 hours

Number of determinations		5
Maximum alkali reserve	31.9 per cent (exper. 96)	
Minimum alkali reserve	19.5 per cent (exper. 68)	
Mean	26.1 volumes per cent	
Maximum fall from normal	40.0 per cent (exper. 68)	
Minimum fall from normal	13.8 per cent (exper. 96)	
Mean fall	22.5 volumes per cent	
Mean heart rate		159
Mean increase in same		53
Mean respiration		62
Mean increase in same		31

Period 4. Ether, 4 hours plus

(See data for experiments 91, 92, 96, table 1)

THE ALKALI RESERVE IN DOGS AFTER TRAUMA UNDER LOCAL ANESTHESIA. NO ETHER

The effects of visceral trauma upon the plasma alkalies when only local anesthesia was used are summarized below in table 2. These experiments were performed by Dr. A. C. Ivy and the writer upon one

TABLE 1

Showing the effects of ether anesthesia upon the alkali reserve of the plasma in dogs

EXPERIMENT NUMBER	CONDITION	ALKALI RESERVE	FALL FROM NORMAL	ARTERIAL BLOOD PRESSURE		HEART RATE	RESPIRATION
				Diastolic	Systolic		
		per cent	*per cent*	*mm.*	*mm.*		
9	Normal	50.7				90	28
	15 min. ether	37.6	13.1			210	70
10	Normal	47.5				90	23
	15 min. ether	40.4	7.1			264	60
11*	Normal	45.7				98	28
	15 min. ether	38.1	7.6			248	60
46	Normal	38.5				104	16
	20 min. ether	37.2	1.3			168	48
44	Normal	45.8				72	12
	25 min. ether	35.6	10.2			132	60
12*	Normal	44.3				102	24
	35 min. ether	38.1	6.2			244	40
90	Normal	40.9				80	6
	38 min. ether	30.5	10.4	146	155	140	56
70	Normal	51.0				60	16
	45 min. ether	36.2	14.8	140	150	132	56
69	Normal	44.9				120	24
	50 min. ether	30.0	14.9	123	131	150	64
29	Normal	36.6				75	24
	55 min. ether	23.3	13.3	102	109	96	72
66	Normal	51.6					
	55 min. ether	34.2	17.4	126	138	122	66
56	Normal	37.6				78	16
	56 min. ether	27.1	10.5	111	118	150	96
31	Normal	49.4				96	15
	75 min. ether	28.7	20.7	94	104	132	44

TABLE 1—*Concluded*

EXPERIMENT NUMBER	CONDITION	ALKALI RESERVE	FALL FROM NORMAL	ARTERIAL BLOOD PRESSURE		HEART RATE	RESPIRATION
				Diastolic	Systolic		
		per cent	*per cent*	*mm.*	*mm.*		
55†	Normal						
	30 min. ether	27.7				204	44
	70 min. ether	16.4		60		174	84
30	Normal	39.3				132	56
	5 min. ether	30.0	9.3			228	66
	75 min. ether	35.3	4.0			180	38
54	Normal	44.9				108	20
	25 min. ether	19.8	25.1			180	52
	90 min. ether	28.2	16.7	115	124	144	76
38	Normal	43.0				72	56
	45 min. ether	31.5	11.5			186	72
	90 min. ether	24.0	19.0	147	153	180	72
60	Normal	40.0				130	80
	50 min. ether	34.3	5.7	124	130	144	108
	85 min. ether	29.6	10.4	118	125	126	104
40	Normal	39.3				108	174
	10 min. ether	31.9	7.4			144	68
	109 min. ether	28.2	11.1	151	154	141	102
43	Normal	44.9				144	48
	45 min. ether	41.9	3.0			144	48
	150 min. ether	32.8	12.1	134	138	192	76
89	Normal	40.4				120	20
	43 min. ether	31.9	8.5	109	121	140	72
	110 min. ether	26.2	14.2	109	125	150	68
14*	Normal	45.3				102	20
	7 min. ether	28.1	17.2			192	92
	56 min. ether	31.9	13.4			162	88
	113 min. ether	29.0	16.3			168	76
67	Normal	41.9				132	35
	58 min. ether	29.3	12.6	92	96	132	56
	120 min. ether	23.7	18.2	94	97	180	38
	180 min. ether	21.4	20.5	55	59	180	64

TABLE 1—*Continued*

EXPERIMENT NUMBER	CONDITION	ALKALI RESERVE	FALL FROM NORMAL	ARTERIAL BLOOD PRESSURE		HEART RATE	RESPIRATION
				Diastolic	Systolic		
		per cent	*per cent*	*mm.*	*mm.*		
68‡	Normal	59.5				84	32
	40 min. ether	35.7	23.8	98	104		
	88 min. ether	27.1	34.4	59	69	132	84
	145 min. ether	19.5	40.0	54	58	180	52
	205 min. ether	22.3	37.2	26	30	102	6
91	Normal	51.0				88	16
	50 min. ether	33.7	17.3	128	131	124	40
	112 min. ether	30.5	20.5	119	122	176	40
	171 min. ether	29.0	22.0	119	121	148	64
	230 min. ether	31.5	19.5	124	126	144	60
	295 min. ether	35.3	15.7	126	128	106	48
	350 min. ether	33.4	17.6	119	122	120	58
	490 min. ether	26.3	24.7	77	79	148	48
	595 min. ether	25.4	25.6	37	38	Dying	
92§	Normal	45.7				108	44
	93 min. ether	30.0	15.7	108	118	120	40
	213 min. ether	28.7	16.0	82	102	128	52
	349 min. ether	24.0	21.7	94	100	128	52
	422 min. ether	26.8	18.9	86	90	164	52
	530 min. ether	26.8	18.9	68	72	160	68
	650 min. ether	30.9	14.8	58	62	176	72
96§	Normal	45.7				120	28
	60 min. ether	34.7	11.0			144	60
	120 min. ether	33.8	11.9			128	48
	240 min. ether	31.9	13.8			160	76
	300 min. ether	33.8	11.9			156	76
	360 min. ether	35.7	10.0			160	88
	420 min. ether	35.7	10.0			168	84
	480 min. ether	35.7	10.0			184	92
	600 min. ether	31.9	13.8			160	120

* Experiments 9, 10, 11, 12 and 14 performed on the same dog.

† Effects of over-etherization shown in experiment 55.

‡ Accidental hemorrhage in experiment 68.

§ Experiments 92 and 96 were aseptic ether controls performed with Mr. C. F. G. Brown assisting. Both dogs lived 12 hours after concluding the experiment.

normal dog and upon four dogs that had had the splanchnics and vagi sectioned in the thorax and the coeliac ganglion evulsated. The operations were performed aseptically two to three weeks previous to the time at which the experiments were conducted, and the dogs had recovered completely.

TABLE 2

Showing the effects of visceral trauma upon the alkali reserve of the plasma in dogs
Local anesthetic. No ether

EXPERIMENT NUMBER	CONDITION	ALKALI RESERVE	FALL FROM NORMAL	ARTERIAL BLOOD PRESSURE, MEAN	HEART RATE	RESPI-RATION	BODY TEMPER-ATURE
		per cent	*per cent*	*mm.*			°C.
78 Type I	Normal	41.9		96	186	16	37.0
	46 minutes*	32.4	9.5	60†	60	12	39.1
77 Type III	Normal	40.9			116	32	39.6
	57 minutes*	35.3	5.6		162	26	39.7
	120 minutes*	23.0	17.9	+	156	20	39.0
79 Type II	Normal	40.0		124	80	14	39.1
	53 minutes*	25.8	14.2	48	144	34	38.7
	93 minutes*	22.1	17.9	42†	124	20	38.6
80 Type I	Normal	37.2		164	140	12	38.3
	33 minutes*	30.9	6.3	48	170	28	38.1
	69 minutes*	29.0	8.2	42	134	32	
	135 minutes*	22.3	14.9	47†	140	32	37.0
76 Type II	Normal	35.3			144	44	38.9
	55 minutes*	30.9	4.4		162	32	37.7
	99 minutes*	24.2	11.1	†	80	12	36.4

* Lapse of time after beginning *initial* trauma.
† Animal in shock at time of observation.

Analyses were made on the plasma of blood drawn by syringe from the inferior vena cava or external jugular vein. The normal dog (exper. 78) was in a state of profound shock at the end of the first forty-five minutes after beginning the initial, and only, period of trauma. As shown in the table the alkali reserve of the plasma had fallen only 9.5 volumes per cent and was in fact no lower than the minimal value for the normal dog. This was a very clear case of type I shock. Of the other four dogs, determinations made before the signs of shock appeared, gave a maximum fall from normal of 14.2 volumes per cent (exper. 79), and a minimum of 4.4 volumes per cent (exper. 76). The average fall for the four, within the first hour after beginning the initial trauma, was 8.1 volumes per cent. As will be seen, the reading in experiment 77 was quite above the normal minimum for the normal dog, namely 32.4 volumes per cent, while the other three gave readings not far below this point.

When we come to the final determinations, however, when all the dogs showed definite signs of shock, matters are somewhat altered. Thus two dogs show a fall from the normal alkali reserve of 17.9 volumes per cent (expers. 77 and 79). The average fall for the four is 15.2 volumes per cent. All show readings below the mean value found after two to four hours of continuous ether anesthesia (26.1 volumes per cent). There appears to be no relation between the type of shock and the degree of alkali depletion. However the data give an indication of what trauma, uncomplicated by ether anesthesia, will accomplish toward setting up a state of acidosis in the dog. It is seen further that until shock actually appears, the alkali reserve remains well above what may be called the limit of safety. The acidosis is certainly not marked. The changes in heart rate and respiration are neither marked nor consistent. As many dogs showed a decrease in both as showed an increase.

It should be emphasized that the marked fall in alkali reserve occurred only after the dogs were in a state of shock. The slight fall observed in the first hour, then, was due to local changes set up by the trauma and not to any circulatory failure. That the onset of acidosis is gradual is revealed in experiment 78, where the dog died from shock before the alkali reserve had fallen even below the minimum normal value.

THE ALKALI RESERVE IN THE ETHERIZED DOG AFTER TRAUMA

The fall in alkali reserve in anesthetized dogs following trauma can best be illustrated by summarizing representative experiments. The data for four such experiments, illustrating the four types of shock, are given in table 3.

The results given in the table above are shown graphically in figures, 2, 3, 4, 5A and 5B.

The data in the above table agree satisfactorily with those presented in table 2. As before, the alkali reserve of the plasma remained prac-

TABLE 3

Showing the alkali reserve after trauma in etherized dogs

EXPERIMENT NUMBER	CONDITION	ALKALI RESERVE	FALL FROM NORMAL	BLOOD PRESSURE		HEART RATE	RESPIRATION	BODY TEMPERATURE
				Diastolic	Systolic			
		per cent	*per cent*					*°C.*
99 Type I	Normal	43.8				132	28	
	62 min. ether	20.2	23.6	153	165	188	64	41.0
	Obs.* 30 min.	21.1	22.7	96	103	160	64	42.0
	Obs.* 95 min.	20.2	23.6	30†				42.0
29 Type II	Normal	36.6				75	24	
	55 min. ether	23.3	13.3	102	109	96	72	
	Obs.* 55 min.	23.3	13.3	64	78	180	48	38.9
	Obs.* 95 min.	17.8	18.8	47	51	180	80	36.8
	Obs.* 127 min.	16.6	20.0	50	53	144	52	35.6
	Obs.* 170 min.	15.7	20.9	29	32†	108	32	
69 Type III	Normal	44.9				120	24	
	50 min. ether	30.0	14.9	123	131	150	64	
	Obs.* 20 min.	27.1	17.8	104	107	180	68	
	Obs.* 78 min.	27.1	17.8	94	98	168	40	
	Obs.* 135 min.	26.2	18.7	67	74	120	36	
	Obs.* 200 min.	15.9	29.0	55	61	126	40	
	Obs.* 255 min.	13.0	31.9	51	58	132	32	
	Obs.* 325 min.	17.8	27.1	20†		52	3	
66 Type IV	Normal	51.6						
	55 min. ether	34.2	17.4	126	138	122	66	
	Obs.* 35 min.	29.4	22.2	126	141	120	72	
	Obs.* 65 min.	30.3	21.3	96	111	150	76	
	Obs.* 115 min.	29.4	22.2	77	84	144	72	
	Obs.* 175 min.	26.5	25.1	78	84	150	78	
	Obs.* 242 min.	22.6	29.0	79	96	192	60	
	Obs.* 310 min.	23.6	28.0	46	53			
	Obs.* 382 min.	32.3	19.3	58	67	126	30	No shock

* Observation taken so many minutes after beginning the initial trauma.
† Observation taken when the animal was in shock.

tically unaffected in this case at the level to which it was brought by etherization, until the blood pressure had fallen; and only showed a significant reduction after the condition of the dog had become serious.

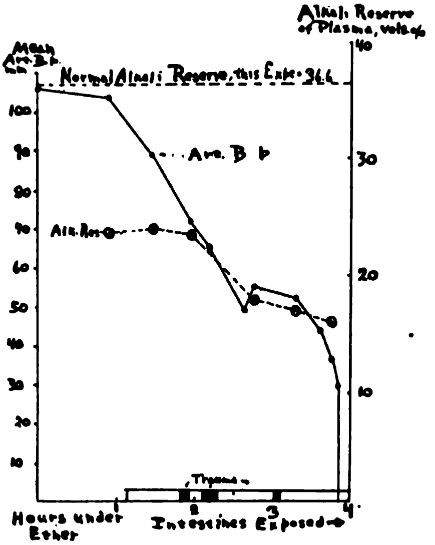


Fig. 2. Experiment 29. Showing the arterial blood pressure and the alkali reserve in a case of type II shock.

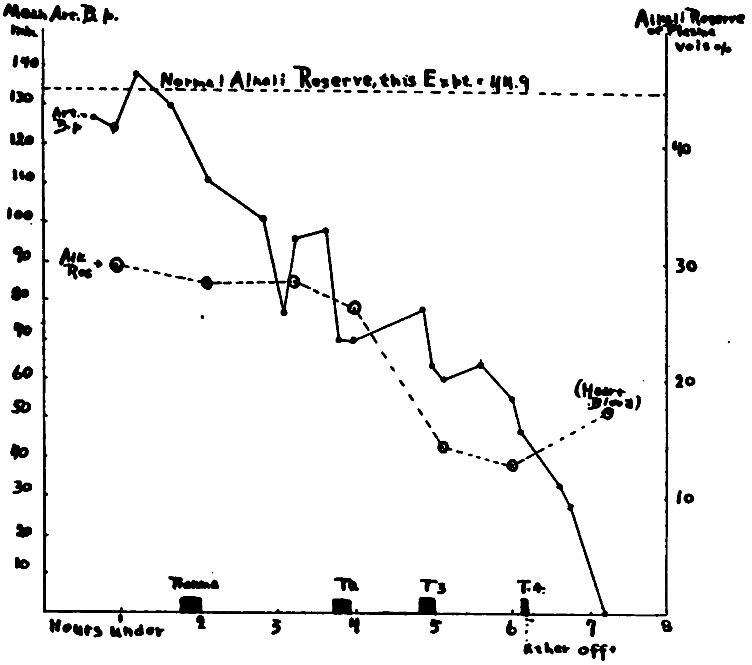


Fig. 3. Showing arterial blood pressure and alkali reserve in experiment 69. Type III shock.

Naturally there were exceptions. In experiment 90 (not shown) for instance, the alkali reserve of the plasma fell to 14.3 volumes per cent while the blood pressure was still 83 to 90 mm. The data also show that as the condition of the dog becomes worse, the heart rate decreases. I have never seen in the dog a case of cardiac shock, as described by Howell (2), (3), result from visceral trauma. At the same time the respiration rate usually decreased. Hence the terminal rise frequently observed in the alkali reserve of the plasma.

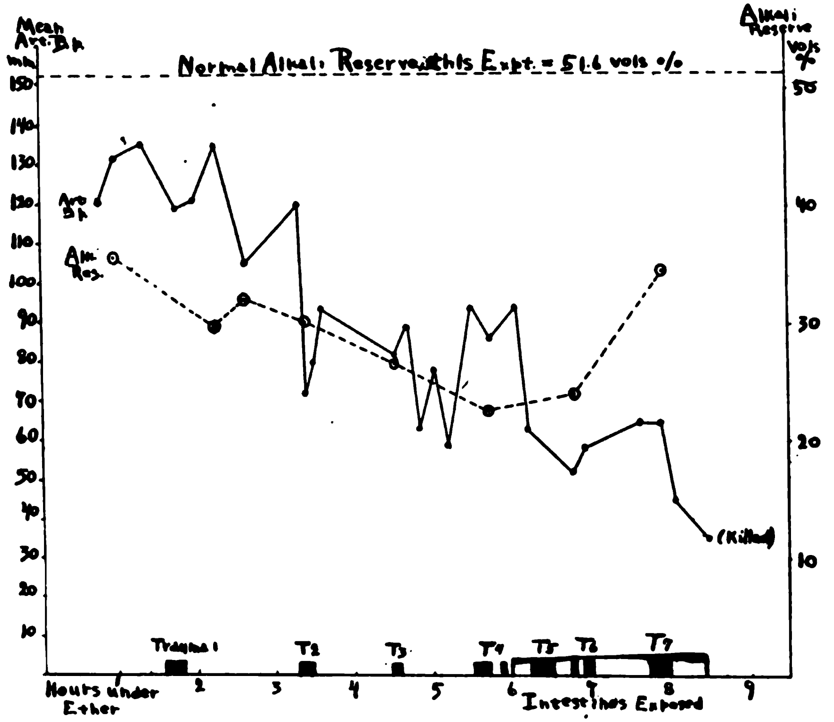


Fig. 4. Showing arterial blood pressure and alkali reserve in experiment 66. Type IV shock.

Although the data tend to show a correlation between the type, *i.e.*, the severity of shock and the fall in the alkali reserve, it appears doubtful, considering the experiments as a whole, whether this is at all close. True, the dogs exhibiting type IV shock were characterized by a remarkably constant alkali reserve. In these dogs compensation, such as by a decrease in the rate of respiration, etc., was no doubt very perfect, even when the blood pressure had fallen markedly. On the other hand, as regards types II and III, the distinction between the two could not be drawn upon the basis of the alkali reserve. Thus the lowest level to which the plasma alkalies were carried by this method, namely 13 volumes per cent (exper. 69, above) was in a case of type III shock.

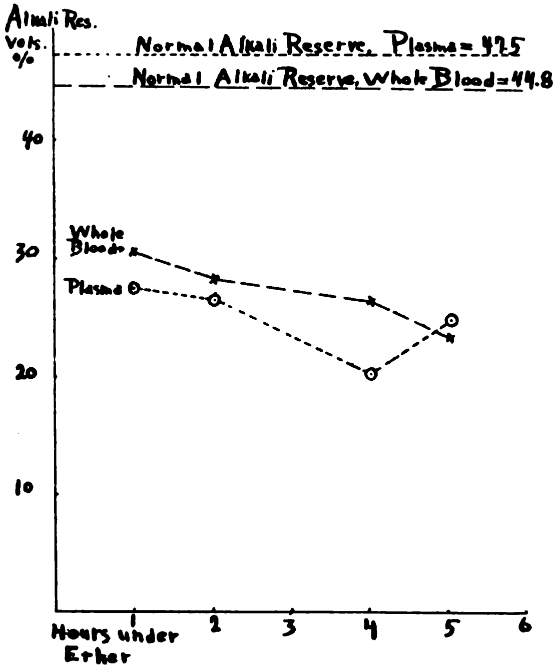


Fig. 5a. Showing alkali reserve of plasma and of whole blood. Aseptic ether control for experiment 99. October 30, 1919.

Again, selecting readings in the first hour after beginning the initial trauma, the alkali reserve in experiment 38 was 18.5 volumes per cent, as compared with 23.3 volumes per cent in experiment 29, although the type of shock in the first case was of the third order while in the second the dog showed type II shock. The blood pressure readings taken at the same time were 94–101 and 64–78 mm. respectively. However, it would be impossible on the basis of the data in hand to assign to any given range of arterial pressures definite values for the plasma alkalies. The venture was made and proved utterly hopeless. Nor is there any critical level of blood pressure at which a marked decline of the alkali reserve is to be expected. The condition of the animal, in short, cannot be gauged by its alkali reserve alone.

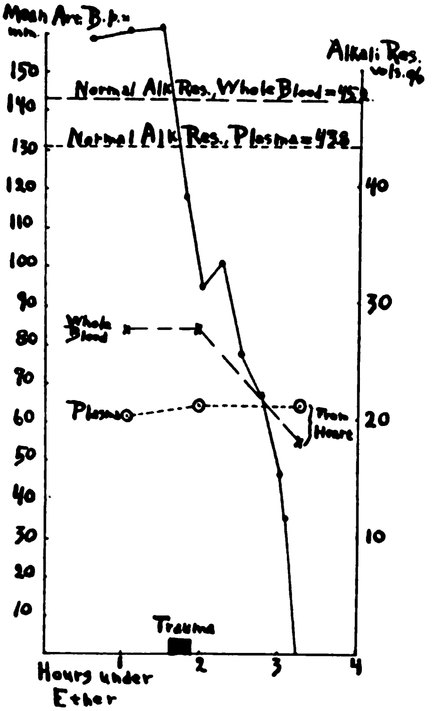


Fig. 5b. Showing arterial blood pressure, alkali reserve of plasma and of whole blood in experiment 99. Type I shock. November 6.

THE ALKALI RESERVE IN CONTROLLED SHOCK EXPERIMENTS

The accuracy of the conclusions reached above can be best borne out by presenting the protocol of one of a series of controlled shock experiments performed with the assistance of Mr. C. F. G. Brown. Dogs were kept under ether anesthesia for considerable periods of time, the anesthetic being administered by intubation. Blood pressure tracings were taken in only one experiment, but blood was drawn from the femoral artery with aseptic precautions at regular and rather frequent intervals. At the close of the experiment the dogs were sewed up and allowed to recover. At the end of a week shock was instituted in the customary manner.

Protocol. Experiment 95, October 31, 1919. Aseptic ether control. Dog 56, male. 15 kilos. Fed.

TIME	ALKALI RESERVE		HEART RATE	RESPIRATION	BODY TEMPERATURE	PROCEDURE
	Blood	Plasma				
a.m.	*vol. per cent*	*vol. per cent*			*°C.*	
8:40	41.0	40.9	96	16		Sample, left saphenous
9:05						Ether, intubated, heat
10:05	28.3	26.8	140	40		Sample, rt. fem. art. Knee jerk positive
11:05	27.4	24.9	140	44		Knee jerk positive
p.m.						
12:05	25.6	25.8	142	36		Knee jerk positive
12:30						Vomited
1:05	28.3	22.1	136	68		Very excited
2:05	28.5	23.0	156	40		Lid reflex negative
3:05	27.5	24.0	152	60		Lid reflex positive
4:05	25.8	22.3	152	36		Lid reflex positive
4:35			140	48	35	Lid reflex positive
5:05	25.7	18.3	156	36		Lid reflex negative
5:35			160	64		Lid reflex positive
6:20					37	Sewed up, ether off

The lid reflex was positive except where otherwise indicated. The dog was under ether 9 hours and 25 minutes. Recovery was good and the dog ate the next morning. After the lapse of a week shock was instituted as shown in the protocol of experiment 100.

The lid reflex was positive throughout the experiment. The ether was disconnected during each period of trauma. On section the heart was found contracted, a further proof, if any were necessary, that in spite of the dog's great excitability the ether had been kept at a minimum level. The total survival time after beginning the initial trauma was three hours and forty-five minutes. Hence according to the classification adopted this was a case of type III shock, there being two periods of trauma, two hours and thirty minutes apart.

It should be noted that the lowest point to which the alkali reserve of the plasma fell, namely 17.7 volumes per cent, was only 0.6 volume per cent below the lowest point reached in the control experiment, and that this value, 18.3 volumes per cent, was perfectly compatible with

Protocol. Experiment 100, November 6, 1919. Shock experiment. Dog 56, male. 10.1 kilos. Not fed.

TIME	BLOOD PRESSURE		ALKALI RESERVE		HEART RATE	RESPIRATION	BODY TEMPERATURE	PROCEDURE
	Diastole	Systole	Blood	Plasma				
a.m.	*mm.*	*mm.*	*vol. per cent*	*vol. per cent*			°*C.*	
9:10			49.4	42.8	80	12		Normal venous sample
9:35								Ether, intubated, heat
10:28	109	127	31.8	31.5	140	44	39.5	Sample, left fem. artery
11:10	101	121	30.8	23.0	144	68	39.0	
11:15	94	113						Trauma, 15 min.
11:30	66	89						Closed up, heat
11:55	84	100			160	68	38.0	
p.m.								
12:10	78	90	29.9	26.8	184	80	38.5	
1:10	78	94	30.7	28.7	156	48	39.3	
1:45	89	109					39.5	Trauma, 15 min.
2:00	61	79			160	32	39.8	Closed up, heat Myenteric reflex positive
2:15	49	63	28.9	17.7	160	36	39.8	
2:25	53	67			176	28	39.0	Ether disconnected, breathing with difficulty, in shock
2:45	26	34	19.7	18.0	96	24	38.5	Respiration spasmodic, "shivery"
3:00								Dead

life. At the time, however, that the low reading was obtained in the shock experiment, the dog was already moribund, with an arterial blood pressure of 49 to 63 mm. Obviously this fact could never have been ascertained by a consideration of the plasma alkali alone. Furthermore the alkali reserve of the whole blood at this time was even slightly higher than in the control experiment at the same hour, namely four hours, forty minutes after beginning ether anesthesia. Bayliss (34) has shown that in cats a reduction of the alkali reserve of the plasma to a level as low as 5 volumes per cent by the intravenous injection of acid does not prevent the quick and apparently complete recovery of the animal. Thus the conclusion is inescapable that there is no critical level of alkali reserve, at least in the dog and cat.

ATTEMPTS TO REPRODUCE TRAUMATIC SHOCK BY INTRAVENOUS INJECTION OF ACIDS AND ACID SALTS

Since the claim put forward by Cannon (11) that the blood pressure in cats can be brought to the shock level by the intravenous injection of N/2 HCl has been effectually disproved (34), it seems unnecessary to give an extended account of the experiments conducted along this line in connection with the present paper.

Intravenous injections of sodium acid phosphate in concentrations of 0.15 to 0.25 molar were made in thirteen dogs and two cats. Five cats and three dogs were given intravenous injections of N/4 HCl. While the injection volume was larger than if half-normal acid had been used, still it is doubtful if there was any significant elevation of blood pressure in consequence of this. Since the acid phosphate was quite ineffective in lowering the alkali reserve of the plasma to any extent, only the results obtained with hydrochloric acid will be given here.

The injection volume used in dogs varied between 6.9 and 42.3 cc. per kilo. All the cats were given 10 cc. per kilo. The lowest point to which the alkali reserve of the plasma was brought in the dog was 11.0 volumes per cent, from an initial normal value of 44.9 volumes per cent. Of this fall 16.7 volumes per cent were due to etherization, and the acid caused a further fall of 17.2 volumes per cent. There were signs of cardiac failure before death and on section the lungs were found to be intensely edematous. Signs of shock were conspicuous for their absence. The blood pressure on beginning the injection was 118–125 mm. After the alkali reserve had fallen to its lowest point the blood pressure remained above 90 mm. for forty minutes and at death was still 57 mm. Fourteen and one-tenth cubic centimeters of N/4 HCl were given per kilo.

The lowest point to which the plasma alkalies fell in the cat was 10.7 volumes per cent, from a value of 34.3 obtained an hour and three quarters after etherization. Of this fall 15.0 volumes per cent were due to the ether. The dose of N/4 HCl was 10 cc. per kilo. The blood pressure was fluctuating but rose from a level of 78–80 mm. at the time the last blood sample was drawn to 81–95 mm. and then fell rapidly to zero. There were no signs of cardiac failure; death occurred in apnoea. There was no hyperpnoea at any time nor any signs of shock, but the possibility of intravascular clotting and embolism was not excluded. Observe that in this cat the alkali reserve was at all times below the critical level of 38 volumes per cent cited by Cannon (11).

Summarizing, it can be said that none of the animals showed any signs of shock; in fact up to the point of death they were all unusually active. There were strong evidences of cardiac failure in the dogs, but whether it occurred in the cats is doubtful. That the oxygenation of the blood was markedly interfered with is shown in experiment 60. After the alkali reserve had been lowered from an initial normal value of 40 to 11.5 volumes per cent, the blood pressure was still 111–126 mm. The injection of acid was continued until the dog had been given 42.3 cc. per kilo of N/4 HCl. The blood pressure fell rapidly from 98–105 to about zero. On drawing a sample of blood from the heart it was found to be quite black and could not be oxygenated at all. The spectroscope revealed reduced hemoglobin only; there was no methemoglobin nor acid hematin. The alkali reserve of the plasma was negligible. In one cat in which death occurred very suddenly on injecting acid, the blood in the right ventricle was found to be coagulated immediately at the close of the experiment.

SUMMARY AND CONCLUSION

1. The dogs used in these experiments are classified on the basis of the length of survival after beginning the initial trauma. This makes unnecessary the arbitrary designation of any given arterial pressure as the shock level.

2. On this basis four general *types of shock* were made out, ranging from the more severe characterized by sudden onset and death, to that in which few or none of the cardinal signs of shock were observed.

3. The larger number of animals showed the intermediate types of shock and lived on the average from two hours, nine minutes, to four hours, fifteen minutes (types II and III) after beginning the initial trauma.

4. The average normal alkali reserve of the venous plasma in the dog was found to be 43.4 volumes per cent, sixty-eight determinations. The values ranged from 32.4 volumes per cent to 59.5. The average for whole blood was 47.0 volumes per cent, eighteen determinations. The maximum reading was 55.6, the minimum 36.6 volumes per cent.

5. In dogs under ether anesthesia the mean value fell to 33.9 volumes per cent. As anesthesia was protracted the mean alkali reserve fell to 28.0 volumes per cent (forty-five minutes to two hours) and finally to 26.1 volumes per cent (two to four hours). As etherization was continued beyond this point the mean fall in alkali reserve per unit of time was seen to decrease.

6. Five dogs traumatized under local anesthesia alone showed no striking fall in the alkali reserve of the plasma until their condition had become quite serious. No level of blood pressure could be set as critical in this respect. Under the conditions of the experiments the average fall from the normal reading was 15.2 volumes per cent.

7. Substantially the same was shown by eleven dogs which were traumatized under ether anesthesia. When shock ensued suddenly the fall in the alkali reserve of the plasma was relatively insignificant. When shock was late in appearing, the plasma might show a high alkali reserve for some time after the animal had become practically moribund. There was apparently no correlation between the type of shock and the degree of alkali depletion. The condition of the animal could not be gauged by its alkali reserve.

8. In several experiments it was found that the alkali reserve of the plasma and of the whole blood fell no lower in profound shock than in aseptic ether controls performed on the same dogs a week previous to the experiment, and from which they recovered rapidly and completely. If there is a critical level of alkali reserve it was not discovered.

9. Intravenous injections of N/4 HCl and of isotonic acid phosphate solutions did not produce shock or anything resembling this condition in either dogs or cats.

The writer desires to thank Dr. A. J. Carlson, Dr. A. B. Luckhardt and Dr. A. C. Ivy for their kind advice and many suggestions, and Mr. C. F. G. Brown for his assistance in the laboratory.

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PHYSICO-CHEMICAL STUDIES ON BIOLUMINESCENCE

III. THE PRODUCTION OF LIGHT BY LUCIOLA VITTICOLLIS IS AN OXIDATION[1]

SAKYO KANDA

From the Marine Biological Laboratory, Kyushu Imperial University, Tsuyazaki (Fukuoka), Japan

Received for publication May 22, 1920

INTRODUCTION

The problem whether the production of light by a fire-fly is an oxidation is an old one, having been raised as long ago as 1783 (2, p. 355). Unfortunately, different investigators offer varying results. Spallanzani, for instance, found that the light produced by Lampyrises disappeared in N_2, H_2 and CO_2, but it appeared again at the admission of air or, better still, O_2. It is doubtful how exact his method was, because it was a work of 1796. On the other hand, according to Macartney, the Lampyris produced a brilliant light without O_2, and it neither became stronger in O_2 nor weaker in H_2 (2, p. 356).

Mangold well points out, therefore, the status of this problem and states:

---

[1] In his paper in this Journal, January, 1920, Doctor Kanda stated that the production of light by Cypridina is not an oxidation. I think it will be admitted that his results were convincing to the extent of showing either that *no* oxygen was required or *very little* (as much as might, in spite of his elaborate precautions, have been present as an impurity). In the interest of clear discussion I believe it should be known that in a personal letter to me, Doctor Kanda now adopts the second alternative. The paper referred to is, I take it, to be interpreted as proving that the luminous material in Cypridina is very rapidly destroyed, without proportional light production, if any considerable amount of oxygen is present; and that the long-continued strong light production which he observed was due to the very small amount of oxygen present as an impurity in the gases used. As Doctor Kanda forwarded his manuscript for publication in English through me, I am venturing to add this note.—E. P. Lyon.

> Die Frage nach der Bedeutung des Sauerstoffes für die Lumineszenz ist hier aber noch nicht . . . . zu einem endgültigen und völlig klaren Abschluss gekommen, zumal noch die letzten Arbeiten über Leuchtkäfer zu scheinbar entgegengesetzten Ergebnissen geführt haben. Die Methodik spielt hier ja eine besonders grosse Rolle, zumal es für einwandfreie Versuche erforerlich ist, so geringe Mengen freien Sauerstoffes auszuschliessen . . . . (2, p. 355).

In short, the problem of oxidation in question is by no means settled. The writer, therefore, made an attempt to settle this problem with new methods and apparatus devised for "einwandfreie Versuche," as Mangold puts it; and he found that the results of these experiments were quite decisive. In this paper, therefore, he will report these results together with the description of methods and apparatus, which were new as far as he is aware. The work was carried out at the Science Department of the Kyushu Imperial University. The writer's thanks are due to Dr. Tsuneya Marusawa, the professor of physical chemistry, for his generous help and suggestions, and also to Mr. Tetsuzo Hagiwara, Doctor Marusawa's assistant, who assisted the writer all the way through the work. The writer appreciates Prof. Ayao Kuwaki's kindness for the privilege of the use of the laboratory.

MATERIAL

The material used for all the following experiments was a Japanese fire-fly, Luciola vitticollis. The luminous organ of this species differs according to the sex. The luminous organ of the male, which is smaller in size than the female, consists of the last two segments of its abdomen, while that of the latter consists of only one segment next to the last.

The luminous organs of the male which were used for all the following experiments, except one series, were carefully cut off from the rest of the body of the live animals. The luminous organs of the female were specially prepared for one series of experiments which were carried out to determine the quantity of oxygen to be consumed by the organs in the oxidation for the production of light. This will be mentioned later on.

THE PREPARATION OF PURE GASES

At first the writer used H_2, N_2 and CO_2 gases which were prepared by ordinary methods and also O_2 from a bomb. The intensity of light produced by the luminous organs of the animals was always strongest and lasted longest in oxygen. The production of light, however, also resulted, though only for a short time, when H_2, N_2 and CO_2 gases were used. On the other hand, no light was produced in vacuum. These peculiar results led the writer to doubt whether the gases used were in reality pure, though they were prepared with special care. The careful analysis of H_2 and N_2 gases with an Orsat's apparatus revealed that they were impure. Their impurity extended even to 1–5 per cent, due to the mixture of O_2. The writer has become convinced since then that gas washers which are used in an ordinary method of gas preparation could by no means purify a gas passed through them, though the air in them was evacuated beforehand.

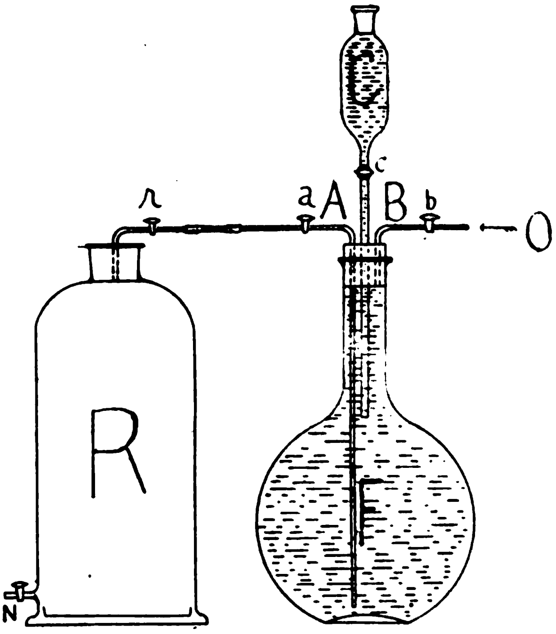

Fig. 1

An exact method, therefore, was to be planned to obviate the failures mentioned above. It was thought that if gas purified by an analytical method could be used, it would serve for this purpose. So a new gas holder was devised, as shown in figure 1. In the first place, the flask, *F*, was filled with a gas-absorbing solution, i.e., 150 cc. of

20 per cent $C_6H_3(OH)_3$ + 800 cc. of saturated KOH for O_2, for example. The solution was drawn in the glass tubes, *A* and *B*, and a separating funnel, *C*, up to the stopcocks, *a*, *b* and *c*. The tube *B* was connected to one of the arms of the Orsat's apparatus by means of a rubber tube. All air in the rubber tube and in the rest of the glass tube *B*, from the stopcock *b*, was evacuated before the connection with the Orsat's was made. A receiving bottle, *R*, which was connected by means of a rubber tube to the glass tube *A* was evacuated and sealed up.

Now 100 cc. of a gas, either H_2 or N_2, which was carefully prepared in the laboratory, were introduced into the burette of the Orsat's apparatus. Oxygen gas which was always mixed as an impurity with H_2 and N_2 gases was repeatedly absorbed by the alkaline pyrogallol solution in a pipette of the apparatus until the volume of the gas in the burette became constant. The gas thus purified was to be preserved in the flask *F* of figure 1. In order that the gas might be drawn into the flask, a stopcock of the arm of the Orsat's, say *O*, to which the flask was connected was to be opened. Thus the rubber tube in the vacuum was now filled with the gas. Then the stopcock *b* was opened. And lastly, the stopcock *a* was opened with careful regulation not to draw the solution in the bottle *R* from the flask *F* too fast. When about all the gas in the burette of the Orsat's was drawn out, all stopcocks just mentioned above were again closed. This same procedure was repeated several times until a desirable amount of the gas had filled the flask *F*. The virtue of this method was that if any traces of O_2 gas were left with the gas in the flask *F*, though hardly possible, the solution in the flask would absorb O_2 during the period of preservation. It is believed that gas absolutely free from even a trace of O_2 gas was available for use by this method.

The oxygen gas taken out from the bomb was also preserved in a flask with a saturated KOH solution in the same way as above after its analysis, although no trace of CO_2 was detected. It was found, however, that the O_2 gas from the bomb was impure to the extent of 2 to 3.25 per cent. Presumably the impurity was nitrogen. No attempt was made to remove the mixed gas or gases from O_2, except CO_2. Carbon dioxide was not used for the experiments of these series because of the difficulty of freeing it from O_2.

METHOD

In the first place, ten isolated luminous organs of the male were placed in the experiment bottle, *E* (fig. 2). The bottle was fitted with a tight rubber stopper in which two glass tubes, *G* and *H*, with one stop-cock for each, were inserted. It was then fixed on an iron stand. The glass tube *G* was connected to the glass tube *A* and the glass tube *H* to one of the arms of a T-shaped glass tube, *J*, as illustrated in figure 2. The experiment bottle, E_1, was also connected to the glass tube, A_1, of the flask, F_1, in the same way as just mentioned above. And one of the arms of the T-shaped glass tube *J* was connected to a Gaede's oil vacuum pump, *P*, through a manometer, *M*. As it was essential to exclude all air from without, melted paraffin was put all over the rubber stoppers of the experiment bottles *E* and E_1, especially those places where the stopper and glass were in contact.

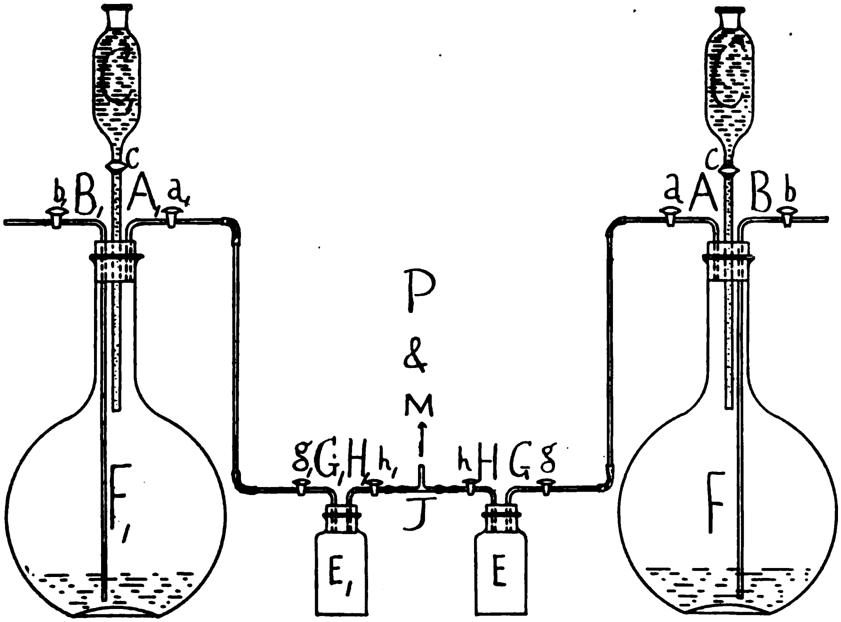

FIG. 2

The vacuum pump was then started to evacuate air in the experiment bottle *E* or E_1. Meanwhile the stopcocks *g* and *H* were opened. When the manometer reached the zero point, the stopcock *H* was closed and the pump was stopped. Of course, some air was still to be left in the bottle and connections[2] because the pressure was not an absolute zero. Nevertheless, the material in the bottle produced no light. In order to make the vacuum of the experiment bottle and other spaces complete, the procedure of evacuation was repeated two or three times after filling the bottle with the pure gas which was to be used. For this purpose, the stopcocks *a* and *c*[3] were opened and the bottle *E* was filled with the gas. After this filling was complete the stopcocks *a* and *c* were closed and the pump was again started to work. This procedure was repeated two or three times. After the last evacuation of the gas, the stopcocks *g* and *h* were closed, and the gas admitted up to the stopcock *g*, opening the stopcocks *a* and *c*. The last procedure of this experimentation was simply to introduce the gas into the bottle *E* to see whether any light was produced by the material or not. But this should be done together with the bottle E_1 which was prepared in the same way.

As O_2 gas was to be used in the bottle, E_1, it was thought that two complete evacuations might be enough. After the last evacuation, the stopcocks g_1 and h_1 were closed and the stopcocks a_1 and c_1 were opened. In so doing, the gas was admitted up to the stopcock g_1. Now both the bottles, *E* and E_1 were ready for the experiment. The room was then darkened. The stopcock g_1 with the left hand and the stopcock *g* with the right were opened at the same time. In this way, O_2 gas into the bottle E_1 and H_2 or N_2 gas, as the case may be, into the bottle *E*, were admitted at the same time. Thus the production of light by the isolated luminous organs of fire-flies in the two gases was observed simultaneously.

The O_2 experiment was always carried-out in comparison with any other experiment as a control of special kind, besides a second control for which air was used. It was thought that the evacuation of air in the experiment bottle might have some effect on the production of light as the result of taking water content away from the material or of some stimulation and other change. Besides the control using air, some experiments were performed by introducing air from a glass gas-holder into the experiment bottle, just the same as the other gases were introduced, after the evacuation of air. These experiments were also compared with those of O_2, as well as with the controls in which air was used without any treatment. It should be remarked that the intensity of light produced by the material was much stronger in air which was introduced *after evacuation* than in air without any treatment. The writer will try to explain this fact later.

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[2] As the Gaede's pump is *capable* of developing a vacuum, 0.001 mm. of Hg., the air left is only $\frac{1}{760,000}$ of one atmosphere after one evacuation. It may be said that there were originally about 12.6 cc. of O_2 in the experimental bottle of capacity of 60 cc., as the amount of O_2 in air is about 21 per cent. There is, therefore, only about 0.0000165 cc. of O_2 left after one evacuation.

[3] The alkaline pyrogallol solution was always poured into the funnel, *C*, by means of a rubber tube connected to the stopcock, *N*, of the bottle, *R*, which was already disconnected from the flask, *F*.

EXPERIMENTAL

As already stated, the purpose of this investigation was to determine whether the production of light by the fire-fly is an oxidation, as is generally assumed, or not. The results obtained prove what most previous authors believed. These experiments were, of course, repeated several times with no exception, when conditions were properly controlled. If there was any exception, it was found that either the gas used was impure, or the method or apparatus imperfect.

The production of light by the luminous organs of the fire-fly in varying gas atmosphere and condition: The methods of these experiments were very simple, as already described in the previous section. Table 1 is the summary of the results of these experiments. The figures in the table simply show a comparative intensity of light produced by the isolated luminous organs of the male in a given gas atmosphere. The figure "4", for example, means that the most intense light was produced by the material in one of the four gases, including air.

As table 1 has shown, no light was produced by the luminous organs in H_2 and N_2 atmospheres or in a vacuum. But the admission of air or better still, O_2 gas, resulted in a brilliant light. Fortunately, once the writer had N_2 gas in which 1 per cent of O_2 gas was mixed. He therefore tried to see whether the material would produce light or not. It was found then that the material produced intenser light in this mixture than in air. The light in the former continued for about 12 hours, while the control in air lasted for about 70 hours at about 20°C. About 3 hours after the extinction of light, the material produced light at the admission of air. These results will convince any unprejudiced minds that the production of light by the material is an oxidation. Furthermore, it is evident that free oxygen is absolutely necessary for an oxidation of this sort, as the material produced no light in vacuum. And it seems therefore probable that even though some oxygen supplier or carrier is assumed to exist in the cells or tissues of the material, it plays no rôle by itself alone in this process of oxidation for the production of light.

The writer stated in the second paper of this series that the production of light by dried crushed Cypridinas was not an oxidation. Some Japanese critics thought that this statement was dogmatic beyond the facts actually found. Their reason was that the writer showed only that no oxygen in the medium was necessary for the production of light by the material, but he did not show at all that oxygen contained in the cells or tissues of the material was not used. The writer could make no answer to this objection. Now it may be answered that it is not probable that any oxygen in the cells, or tissues of the dried crushed Cypridinas is used for the production of light by them, because no oxygen in the cells or tissues of the luminous organs of the fire-flies seems to be used for their production of light, even though this process is certainly an oxidation.

TABLE 1

The production of light by the isolated luminous organs of Luciolas in varying gas atmosphere and condition

GAS AND CONDITION	INTRODUCTION OF O_2 AFTER EVACUATION	INTRODUCTION OF AIR AFTER EVACUATION	INTRODUCTION OF N_2 + 1 PER CENT O_2 AFTER EVACUATION	AIR CONTROL	INTRODUCTION OF H_2 AFTER EVACUATION	INTRODUCTION OF N_2 AFTER EVACUATION	VACUUM
Comparison of intensity of light.	4	3	2	1	0	0	0
Readmission of air.............	4	2	2 (?)	1	3	3	3

As already stated, the light produced by the material in air admitted after one evacuation was much stronger than that produced in air without any treatment. This fact could not be explained on a mere basis of the volume of oxygen contained in air. But as the cells and tissues of the material were alive and some nerve ganglia seemed to be located in the tissues, it seemed possible that stimulation by mechanical agitation occurred when air was admitted after an evacuation. This might be the same phenomenon as in the case of the fire-flies producing a stronger light when water is sprinkled on the cage containing them, or when the cage is shaken. Besides such biological factor, the surface of contact may also act. Evacuation may increase the contact-surface of the material for the readmitted oxygen gas and in consequence the rate of oxidation may increase. Whatever the reason, the result was a stronger light when air was readmitted after evacuation. The fact that the intensity of light produced by the material in the gaseous mixture of N_2 with 1 per cent of O_2 admitted after evacuation was stronger than that of light produced in air with no evacuation may also be explained in the same way. That the light produced by the material in air which contains about 21 per cent of O_2 is weaker than that in the mixture of N_2 with 1 per cent of O_2 is incomprehensible if considered merely from the viewpoint of an oxidation. But it is not necessarily so if the biological and physico-chemical factors, mechanical agitation and surface action just mentioned above, are considered.

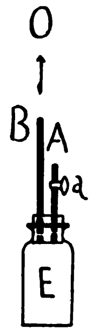

FIG. 3

An estimation of oxygen consumed by the luminous organs: An attempt was made to estimate the amount of O_2 converted into CO_2 in the production of light by the isolated luminous organs of the female. A preliminary experiment showed that quite a large amount of CO_2 was given off during the production of light by the luminous organs. This encouraged the writer to undertake further careful experiments. The method of this series of experiments was a little different from others, though quite simple. The bottle shown in figure 3 was used for this experimentation.

In the first place, the experiment material was isolated as carefully as possible to minimize the admission of other substances, which might in some way obscure the results. For this purpose the luminous organ of the female was more suitable than that of the male, because the female luminous organ of this species consists of only one abdominal segment next to the last, as already stated. If the thoracico-abdominal regions were pressed by the fingers of the left hand, the last two or three segments of the abdomen were stretched out. Then the last segment was carefully cut off by means of sharp scissors and the thoracico-abdominal regions were again pressed hard. In doing so, eggs and other matter contained in the abdomen were pressed out from the cut. They were all cleaned off with special care. After this cleaning, the luminous segments of sixty females thus isolated were placed in the experiment bottle *E* shown in figure 3. The bottle *E* was tightly fitted with a rubber stopper in which two capillary glass tubes, *A* and *B*, were inserted. The tube *B* was connected by means of a rubber tube to one of the arms of the Orsat's apparatus for gas analysis. The tube *A* which had one stopcock, *a*, was connected to the vacuum pump through a manometer. After all connections were thus made, melted paraffin was put all over the stopper of the bottle *E* as usual. Now the stopcock of the arm of the Orsat's, say *o*, to which the tube *B* was connected, was closed. The pump was started to evacuate the air in the bottle *E*. After complete evacuation, the stopcock *a* was closed.

Exactly 100 cc. of O_2 gas, which contained about 2.14 per cent N_2 but was absolutely free from CO_2 and was held in the burette of the Orsat's, were ready to be sent into the bottle *E* at any time. Now the stopcock *O* was opened. Oxygen gas was thus introduced into the bottle *E* from the burette. After this filling was over, the stopcock *O* was closed. The exact volume of the gas introduced into the bottle was read and the rest of the gas left in the burette was blown out. At the same time the barometer and temperature of the laboratory were read. For certain hours the material thus treated was allowed to use O_2 for the production of light, the exact volume of which was already known.

Twenty-five hours from the time of treatment the material was still producing light. But it was thought that further delay might complicate the results by increasing CO_2 gas which might be given off due to the decay of the material, or to other causes. Therefore the tube *A* was connected to a syphon by means of which distilled water was caused to run into the bottle *E*. The stopcocks *O* and *a* were opened in the order mentioned. The gas contained in the bottle was thus displaced by the water and returned to the burette. When the water reached the stopcock *O*, the latter was closed. The volume of the returned gas and the temperature and barometer were read at the same time. The carbon dioxide mixed with the O_2 gas was absorbed by a concentrated KOH solution until the gas volume became constant. The O_2 was also absorbed by the alkaline pyrogallol solution after an addition to it of 16 cc. of N_2. The results of the experiments calculated by reducing to the normal conditions are given in table 2.

The amount of O_2 consumed in the production of light by the material was found to be 6.01 cc., as shown in table 2. But the methods of the experiment were not beyond criticisms, which may be mentioned as follows: *a*, The gas in the burette of the Orsat's apparatus was displaced by distilled water. It was certain that the water in the burette would dissolve CO_2 which was sent for analysis from the experiment bottle into the burette, after the luminous organs had used O_2 for the production of light. An error would thus be introduced into the results. In other experiments, therefore, the water in question was replaced by mercury before the gaseous mixture of O_2 and CO_2 was sent back into the burette. Unfortunately, however, a certain technical fault was found in the process of analysis. So these results have been ignored and the experiments made with water displacement are published. *b*, It was not at all certain whether the whole amount of O_2, i.e., 6.01 cc., was exclusively used up for the production of light by the isolated luminous segments, as some of it might have been used for oxidation of substances of the segments independent of the process of the light production.

The writer was anxious to remove as far as possible these errors and objections just mentioned. It was, however, late in the season and the material could not longer be secured. He therefore reports the result as it was, though unfinished.

TABLE 2

An estimation of O_2 consumed by the isolated luminous organs of Luciolas for 25 hours

O_2 ORIGINALLY USED	$O_2 + CO_2$ AFTER 25 HOURS	DEVELOPED CO_2 FOUND	O_2 REMAINING	CONSUMED O_2 FOUND
cc.	*cc.*	*cc.*	*cc.*	*cc.*
58.52	58.17	5.66	52.51	6.01

A relation of the production of light to water: It was supposed that if the production of light by the material was an oxidation, the material might be preserved longer in vacuum than in air. So the following experiments were tried. Seventeen glass tubes of the capacity of about 25 cc. were drawn narrow at one end and sealed up at the other end. Isolated luminous organs of ten males were placed in each tube. Three of these tubes were sealed up at a certain time. These were the controls of one kind. Another three tubes which were thoroughly evacuated once were sealed up after readmitting air. These were controls of another kind. The material in the controls of these two kinds was of course producing light. The rest of the tubes were sealed up while the process of evacuation was going on. The material in these tubes was not producing light. At an interval of 2 or 3 hours, one of these tubes was opened in the dark room to see whether the material produced light or not. In doing so it was thought that the exact time might be detected at which the material no longer produced light. But as table 3 has shown, the results were contradictory and were quite contrary to the writer's expectation. That is to say, the material was preserved longer in the air than in the vacuum.

The material of the controls of the second kind which were evacuated and were sealed up after admission of air, seemed to furnish an explanation to the riddle. There should be no difference in the durability of light between the first and second controls because the volume of air in both was practically equal. But perhaps the material which was temporarily exposed to a vacuum might have lost some water during the process. The loss of water might perhaps have shortened the endurance of the light-producing substance. This view may also explain the failure of experiments in which all the material was sealed up for a long time in the vacuum tubes. It may be asserted as probable, therefore, that water is necessary for the production of light by the fire-fly.

TABLE 3

The production of light by the isolated luminous organs in air admitted after the enclosure in vacuum

CONDITION TIME IN HOURS	PRODUCTION OF LIGHT BY THE ISOLATED LUMINOUS ORGANS SEALED IN AIR	PRODUCTION OF LIGHT BY THE ISOLATED LUMINOUS ORGANS SEALED IN AIR AFTER EVACUATION	PRODUCTION OF LIGHT BY THE ISOLATED LUMINOUS ORGANS SEALED IN VACUUM	PRODUCTION OF LIGHT BY THE ISOLATED LUMINOUS ORGANS SEALED IN VACUUM AT THE ADMISSION OF AIR
1	+	+	–	+
5	+	+	–	+
10	+	+	–	+
15	+	+	–	+
20	+	+	–	+
25	+	+	–	+
30	+	+	–	–
35	+	+	–	–
40	+	–	–	–
45	+	–	–	–
50	–	–	–	–

This view is strengthened if the following fact is considered. That is to say, dried crushed luminous organs of the fire-fly produce light, though faint, if moistened. The fire-fly seems, however, to be quite different from Cypridinas. The more dried the longer the latter is preserved, while the dried fire-fly or its luminous organ is preserved only 5 or 6 days. That is to say, the dried luminous organ of the fire-fly did not produce light after 5 or 6 days even though moistened. A question arose whether the luminous organs of fire-flies produced light when they were dead if moistened or not. And absolute dryness of the organs in question might be one of the causes of death. This idea was tested but no decisive results were obtained.

The effect of temperature: Harvey states that "Luciola photogenin is destroyed at about 42°, while the photophelein is still active after ten minutes boiling" (1, p. 348). The writer found that the light produced by the isolated luminous organs of Luciola vitticollis disappeared when heated at 50°C., but it returned again when cooled. The return of light took place after about 5 or 10 minutes and it was very faint.

SUMMARY AND CONCLUSION

1. The material used for experiments was a Japanese fire-fly, Luciola vitticollis.
2. The gases used for experiments were H_2, N_2 and O_2.
3. New methods and apparatus were contrived to purify and manipulate the gases to fit the purposes of this investigation.
4. The isolated luminous organs of the animals produced no light in H_2 and N_2 or in vacuum. The oxygen of the cells or tissues of the organs, therefore, seemed not to be used for the production of light.
5. The intensity of light produced by the isolated luminous organs was greatest in O_2 atmosphere, next in air which was introduced after evacuation, then in N_2 mixed with 1 per cent of O_2 and last in air.
6. The isolated luminous organs of sixty females which were placed in 58.52 cc. of O_2 gave off 5.66 cc. CO_2 in 25 hours. The amount of O_2 consumed was 6.01 cc.
7. Water seemed to be necessary for the production of light by the isolated luminous organs.
8. The light produced by the isolated luminous organs disappeared when heated to 50°C., but it appeared again when cooled.

The principal conclusion on the basis of the experimental results mentioned above is that the production of light by Luciola vitticollis is an oxidation.

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BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA

I. MIXED DIET REACTION

G. H. WHIPPLE, C. W. HOOPER AND F. S. ROBSCHEIT

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

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This series of papers deals with the regeneration of red cells and hemoglobin following simple anemia and the influence of diet factors upon this reconstruction. It will be shown that the curve of hemoglobin regeneration can be influenced at will by various diet factors. We believe that it is desirable to mention at least two lines of investigation which are being followed in this laboratory. To determine the value of various food factors when given alone or combined with other substances. To determine further the few or many substances which promote speedy regeneration of hemoglobin and red cells or to ascertain the optimum food combinations which will give a maximum blood regeneration following simple anemia. Inorganic substances and certain drugs are being investigated and this work will be presented in its proper place. Some experiments will deal with splenectomized dogs as well as bile fistula dogs but we prefer to present at a later time the bulk of our work on splenectomized animals which deals with the relation of splenectomy to blood regeneration under fixed experimental conditions. A preliminary report covering a part of this anemia work has been published elsewhere (1).

This work on blood pigment regeneration forms an essential part in any study of "pigment metabolism of the body." It is obviously closely related to a study of *bile pigment excretion* which was first taken up in our work and has been reported in part in earlier publications (2), (3). It will be recalled that the excretion of bile pigments may be influenced or modified by various diet factors. For example, meat will cause increased flow of bile but a decided drop in total bile pigments. Carbohydrate, on the contrary, will reduce the flow of bile but increase the total bile pigment output. Decreased functional activity of the liver is associated with a decided fall in bile pigment elimination. We have assumed on the basis of much experimental evidence that the liver plays a *constructive* rôle in the bile pigment output. We hope to show the same relationship on the part of the liver to the *constructive mechanism* of blood regeneration.

We wish to emphasize that a curve of blood pigment regeneration cannot be established without accurate determination of at least two factors,—hemoglobin and blood volume. With a knowledge of these two factors we can estimate the total volume of hemoglobin pigment in the body circulation—the "pigment volume." Also with the hematocrit values we are able to compute the total volume of red cells in the body circulation. Reasonably accurate methods for the determination of circulatory blood volume are of recent development. A critical review of many factors in this blood volume work and comparison of various methods have been published from this laboratory very recently (4). The method used in this laboratory for accurate determinations of hemoglobin has been recently described by F. S. Robscheit (5).

Practically all these anemia blood regeneration experiments were performed upon dogs born and raised in our kennels—a bull dog cross which gives a very active, vigorous and healthy laboratory animal. Unless otherwise noted, the dogs were in fine normal condition during the entire experiment. These dogs will eat the food mixtures as a rule without any delay or wastage. They have been immunized against distemper and are kept in a separate room to obviate any cross infections by transient animals.

METHODS

The blood volume method used in these experiments has been described in detail elsewhere (4). In some of the earlier experiments dry oxalate was used for blood collection instead of isotonic fluid oxalate and in these experiments the calculated blood volume is too high. A note will be made in all such experiments as a correction cannot be introduced because the amount of solid oxalate and the corresponding shrinkage of cells was an unknown variable.

It may be stated in a word that the blood volume method consists in the introduction of a measured amount of a dye "brilliant vital red" into the blood stream. After a four minute period the dilution of the dye in the plasma is colorimetrically determined. "Brilliant vital red" has been furnished us through the courtesy of Dr. H. M. Evans of the Department of Anatomy and we wish to acknowledge many favors and valuable advice given us by Doctor Evans. The red cell hematocrit is read in an accurately calibrated centrifuge tube into which blood has been drawn, using an isotonic sodium oxalate solution. It is then a simple matter to calculate the plasma volume, red cell volume and total blood volume. This method can be quickly and accurately performed. It causes the dog a minimal degree of inconvenience, only that due to a hypodermic needle puncture of a vein, and the loss of only 35 cc. of whole blood. This is such a small amount of blood removed from the large circulating blood volume that we do not include it in our calculations and feel that no secondary anemia factors are added to complicate the reaction curve following the initial bleeding.

The hemoglobin determinations are made by means of a modification of Palmer's method, recently described in detail by one of us (5). This insures an accurate measure of the hemoglobin, as relatively large amounts of packed red cells are used. In some of the earlier experiments the Sahli hemoglobin tubes were used and in some instances these tubes had faded, giving hemoglobin values which were too high. The base line of any experiment although too high will not disturb the fairly accurate curve of regeneration which is more important. A footnote will be appended to all experiments in which the Sahli readings are given.

Red and white cell counts are made in the routine manner. Identical counts have been repeatedly obtained by venous puncture and from a freely bleeding ear puncture. The former is now our routine procedure.

The simple anemia is produced in the following manner: A simple blood volume is performed. The next day the dog is bled one-fourth the determined blood volume. This is easily done by inserting a needle into the jugular vein and aspirating the blood into a calibrated flask containing oxalate. The following day the same amount of blood is aspirated in exactly the same manner. During these two bleeding days and the next resting day the dogs are on a bread and milk diet. Following the resting day a second blood volume is done to determine the actual amount of anemia produced. The calculated and actual figures do not correspond but too many factors enter this equation to permit a discussion at this time: for example, the reserve of blood cells thrown in from the marrow, to mention only one. At times a third bleeding is necessary if the reserve has been too great. A third blood volume is then done. The dog is then placed upon a fixed diet and complete blood volume, hemoglobin and blood cell determinations are done once each week thereafter. Special care is taken to insure a sufficient food ingestion based on the number of calories and nitrogen intake. The weight curve is a good index of the general nutrition.

The dogs are kept in individul cages which are comfortable and of suitable size to permit of much exercise. The cages are cleaned once daily by the attendant, but all feeding is done by the person in charge of the experiment. The mixed foods are usually eaten at once when placed each morning in the cage. Fluids are usually given by stomach tube. Water is furnished in the cage at all times except in metabolism experiments when it is usually given by stomach tube. It should be emphasized again that these dogs were raised in the laboratory and are therefore healthy, vigorous and very active at all times. The laboratory routine disturbs them not at all and the performance of the blood volume requires only a few minutes. They will eat all manner of food mixtures with relish and alacrity. Unless otherwise noted, these dogs are in their usual healthy, active condition throughout the entire experiment. "Mixed diet" in these experiments indicates a mixture of food materials obtained from the University Hospital, consisting of bones, bread, cooked meat, potato, rice, macaroni and general table scraps.

EXPERIMENTAL OBSERVATIONS

The dogs used in most of the experiments tabulated below are young animals in the active growth period,—one year of age or less. Such animals are increasing in size, weight and strength and the demand for tissue building or growth factors is acute. It is therefore of interest to keep this fact in mind during our analysis of the subsequent experiments. It might be assumed that the reconstruction of red cells in a rapidly growing animal might be handicapped by the tissue demand for normal growth factors. On the other hand, it may be argued that the growth capacity of the younger organisms might be greater as regards the construction of tissue cells, including the red cells. When we review the experiments to be submitted in subsequent communications it may be stated that the difference between the adult and the young dog is not great as regards the capacity of the animal to regenerate new red blood cells. This statement applies to young dogs between 6 and 12 months of age and takes into consideration the individual variations which are met with in different dogs. It is possible that this statement may not hold for pups less than 6 months of age. In general we may be safe in stating that if there is any difference between young and adult dogs, there is a slight difference in favor of the adult dogs who at times seem to show a slightly shorter period of blood regeneration under similar circumstances.

TABLE 1

Blood regeneration—mixed diet. Dog 19–93. Bull pup, female, age 13 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/11	1780	1280	580	688	53.7	139	0.91	7,6	16,2	12.6	101	
8/12	Diet: Bread and milk											
8/12	Bled 320 cc. Slight distress. Injection 100 cc. N/1 salt solution											
8/13	Bled 320 cc. No distress											
8/15	614	986	732	249	25.3	62	0.97	3,2	12,8	13.0	76	Normal
8/15	Diet: Mixed diet											
8/21	658	1023	716	292	28.5	64	0.80	4,0	6,2	13.25	77	Diet poor in meat
8/28	962	1210	728	465	38.4	79	0.69	5,7	10,8	13.3	91	
9/4	1280	1308	724	572	43.7	98	0.82	6,0	10,0	14.1	93	More meat in food
9/11	1538	1420	673	733	51.6	108	0.64	8 4	8,8	14.3	99	
9/18	1612	1355	644	700	51.6	119	0.68	8,7	10,4	14.5	94	
9/25	1726	1444	681	748	51.8	120	0.69	8,7	12,6	15.5	93	
10/2	1830	1490	675	793	53.2	123	0.72	8,5	12,0	15.7	95	

The first three experiments (tables 1, 2 and 3) were performed upon three young dogs of the same litter, all of the same weight, activity and general appearance. It will be observed that there are individual differences even under these favorable conditions. It is possible that some of this variation may be explained by individual preferences of the different dogs for various food factors. They were all given an excess of mixed food from which of course they could pick out the bits of food which they preferred. During much of this diet experiment the "mixed food" was poorer than usual in meat and bones. This explains the fact that the period of blood regeneration appears somewhat longer than in some of the subsequent experiments. The explanation for this fact is found in paper IV of this series, which shows the remarkable efficiency of a meat diet in promoting blood regeneration.

TABLE 1-B

Experimental history. Dog 19-93

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 1/16/19	Rice, potato, milk	1435	126	10.95	Born July 11, 1918
Bled 590 cc.		594	86	10.25	
End 3/7/19		1098	113	10.35	Maximum regeneration 5 weeks
Begin 3/17/19	Rice, potato, milk (repeat)	1040	89	11.35	Table 42
Bled 502 cc.		566	82	11.15	
End 4/30/19		1313	107	11.15	Complete regeneration 4 weeks
Begin 8/11/19	Mixed diet	1780	101	12.6	Table 1
Bled 640 cc.		614	76	13.0	
End 10/2/19		1830	95	15.7	

It is to be noted that all these three dogs increased markedly in body weight. It should be stated that this was a general growth with increase in size, length of limb and body, not merely a deposit of fat. One is not surprised to note a gradual increase in blood and plasma volume during the experiment. The total volume of red cells, hematocrit reading of red cells and hemoglobin follow curves which are parallel. The color index is almost constantly between 0.65 and 0.85 and there is not a very strong tendency in these experiments for the color index to drop much below normal in the first two weeks following the hemorrhage. We shall not attempt at this time to discuss the fluctuations in white blood cells which are tabulated.

The "pigment volume" is a convenient and expressive term which indicates the total volume of circulating or effective blood pigment in the blood stream at the time of estimation of blood volume and hemoglobin. The *pigment volume is the product of blood volume times per cent hemoglobin.* In all tables the *pigment volume* is the first value given as we believe it gives the best general index of the curve of blood regeneration.

TABLE 2

Blood regeneration—mixed diet. Dog 19–94. Bull pup, female, age 13 months

DATE, 1910	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cen*				*kgm.*	*cc.*	
8/11	1705	1242	572	658	53.0	137	0.78	8,8	7,6	11.0	113	
8/11	Diet: Bread and milk											
8/12	Bled 310 cc.											
8/13	Bled 310 cc. No distress											
8/15	540	1000	761	230	23.0	54	0.84	3,2	6,4	13.5	74	Normal
8/15	Diet: Mixed diet											
8/21	686	1090	768	306	28.1	63	0.67	4,7	8,6	13.65	80	Diet poor in meat
8/28	800	1194	812	370	31.0	67	0.78	4,3	6,0	13.4	89	
9/4	972	1292	843	443	34.3	75	0.66	5,7	12,0	13.9	93	More meat in diet
9/11	1032	1328	836	489	36.8	78	0.67	5,8	8,2	14.0	95	
9/18	1062	1205	736	465	38.5	88	0.69	6,4	6,2	13.8	87	
9/25	1226	1295	760	524	40.4	95	0.68	7,0	7,8	14.45	90	
10/2	1298	1276	710	554	43.4	102	0.74	6,9	17,4	14.55	88	More meat in diet
10/9	1520	1463	827	624	42.6	104	0.73	7,1	13,6	15.5	95	
10/23	1550	1463	823	620	42.4	106	0.74	7,2	8,8	16.25	90	

Table 2, dog 19-94 is an experiment with a dog presenting some unknown abnormal factor. This dog at times shows an eosinophilia yet only an occasional parasite egg can be demonstrated in the feces.

Treatment by oil of chenopodium and santonin has yielded no results. The dog is not as well nourished as the others of this litter and at times presents a slight relative degree of anemia. This fact is to be considered in a study of the abnormally slow blood regeneration in this dog. In spite of this the dog gained about eleven pounds during the course of the experiment.

Table 3 at the start of the experiment shows the remarkably high figure (152 per cent hemoglobin) which may be observed in normal dogs. Red blood counts of 7 to 9 millions are the rule.

TABLE 2-B

Experimental history. Dog 19–94

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 1/16/19	Bread, 300 grams, milk 500 cc.	1250	126	10.65	Born July 11, 1918
Bled 620 cc.		462	84	10.00	
End 3/7/19		899	94	11.70	Maximum regeneration 5 weeks
Begin 3/17/19	Bread, 300 grams, milk 500 cc. (repeat)	1146	93	13.05	Table 27
Bled 608 cc.		542	78	12.40	
End 4/30/19		1031	99	13.20	Maximum regeneration 5 weeks
Begin 8/11/19	Mixed diet	1705	113	11.00	Table 2
Bled 620 cc.		540	74	13.5	
End 10/23/19		1550	90	16.25	

October 9. Two doses of oil of chenopodium, 48 hours apart. Few ova found. No worms expelled. Last dose followed by santonin mixture. No effect.

Table 4 shows an experiment upon a young dog (6 months) in which one week's diet of rice, potatoes and milk followed the bleeding—then the usual mixed diet. The dog did not eat the rice, potato and milk diet and lost much weight. Also she developed signs of mild distemper, but this soon cleared up after being put on a liberal mixed diet. There was then a rapid gain in weight as well as in blood regeneration. The leucocytosis is probably to be explained by the mild infection with distemper. The total regeneration of red cells is complete in one month.

TABLE 3

Blood regeneration—mixed diet. Dog 19–95. Bull pup, female, age 13 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	*per cent*	*per cent*				*kgm.*	cc.	
8/11	2088	1369	568	794	58.0	152	0.88	8,6	10,4	13.2	104	
8/11	Diet: Bread and milk											
8/12	Bled 342 cc.											
8/13	Bled 342 cc. No distress											
8/15	750	1005	700	300	29.8	74	1.03	3,6	8,4	13.65	74	Normal
8/15	Diet: Mixed diet											
8/21	843	1106	728	367	33.2	76	0.93	4,1	17,8	14.2	78	Diet poor in meat
8/28	829	1066	747	414	35.5	78	0.75	5,2	10,2	14.0	83	
9/4	954	1243	814	423	34.0	77	0.71	5,4	19,0	14.65	95	More meat in diet
9/11	1313	1338	726	600	44.8	98	0.65	7,5	13,2	14.5	92	
9/18	1325	1243	674	552	44.4	107	0.74	7,2	16,0	14.65	85	
9/25	1730	1412	678	720	51.0	122	0.74	8,2	13,0	15.25	93	
10/2	1874	1410	618	779	55.2	133	0.75	8,8	16,4	15.45	91	
10/9	1655	1343	654	676	50.3	123	0.66	9,0	14,4	15.70	86	

TABLE 3-B

Experimental history. Dog 19–95

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 1/16/19	Rice, potato, milk	1232	100	11.35	Born July 11, 1918
Bled 564 cc.		560	75	11.00	
End 3/7/19		983	90	11.20	Complete regeneration 5 weeks
Begin 3/17/19	Rice, potato, milk (repeat)	1092	88	12.80	Table 41
Bled 566 cc.		570	75	12.45	
End 4/30/19		1237	98	11.95	Complete regeneration 5 weeks
Begin 8/11/19	Mixed diet	2088	104	13.20	Table 3
Bled 684 cc.		750	74	13.65	
End 10/9/19		1655	86	15.70	Maximum regeneration 7 weeks

TABLE 4

Blood regeneration—mixed diet (following rice, potatoes and milk). Dog 19–96. Bull pup, female, age 6 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.	
1/16	1200	1025	483	540	52.3	117	0.58	10,1	20,8	10.15	101	
1/16	Diet: Crackermeal and milk											
1/17	Bled 256 cc.											
1/18	Bled 256 cc. No distress											
1/20	514	778	574	196	25.2	66	1.10	3,0	16,0	9.75	80	Normal
1/20	Diet: Boiled rice, 200 grams; potatoes, 200 grams, milk, 500 cc.											
1/27	422	826	604	218	26.4	51	0.48	5,3	12,4	8.60	96	Mild distemper
1/27	Changed to mixed diet*											
2/3	765	915	554	357	39.0	84	0.67	6,3	15,8	10.1	91	Recovered from distemper
2/12	1013	1080	574	500	46.3	94	0.65	7,2	12,0	11.35	95	
2/19	1243	1243	646	584	47.0	100	0.71	7,0	12,8	11.80	105	
2/28	1288	1108	537	554	50.0	116	0.73	8,0	9,2	11.95	93	

* Developed mild case of distemper. Refused to eat rice and potatoes.

TABLE 4-B

Experimental history. Dog 19–96

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 1/16/19	Rice, potato, milk for 1 week. Mixed diet for 4 weeks	1200	101	10.15	Born July 14, 1918
Bled 512 cc.		514	80	9.75	
End 2/28/19		1288	93	11.95	Table 4
Begin 3/17/19	Rice, potato, milk	1520	92	13.50	Table 44
Bled 620 cc.		527	72	12.40	
End 4/30/19		778	95	9.90	Maximum regeneration weeks
Begin 8/11/19	Mixed diet	1750	98	13.65	Table 5
Bled 668 cc.		650	72	13.35	
End 10/2/19		2012	98	15.45	

Table 5 shows a second experiment on the same dog, 19–96, given in table 4. The first experiment was done at the age of 6 months and this experiment at the age of 13 months. The period of blood regeneration is longer in this experiment as we believe the correct explanation is the low meat content of the mixed diet during this period. This was also noted in tables 1, 2 and 3, which experiments were all done at the same time. Our experiments are usually carried out in groups of four.

TABLE 5

Blood regeneration—mixed diet. Dog 19–96. Bull pup, female, age 13 months

DATE, 1910	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/11	1750	1335	658	670	50.2	131	0.90	7,3	14,6	13.65	98	
8/11	Diet: Bread and milk											
8/12	Bled 334 cc.											
8/13	Bled 334 cc. No distress.											
8/15	650	964	684	275	28.5	67	0.90	3,7	21,6	13.35	72	Normal
8/15	Diet: Mixed diet											
8/21	660	993	686	297	29.9	66	0.80	4,1	12,0	13.9	71	Diet poor in meat
8/28	801	1155	770	368	31.9	69	0.66	5,2	12,4	14.05	82	
9/4	1054	1340	852	474	35.4	79	0.72	5,5	16,2	14.7	91	More meat in diet*
9/11	1255	1340	742	596	43.7	94	0.64	7,3	18,2	14.65	91	
9/18	1348	1225	639	575	46.9	110	0.65	8,5	10,8	14.85	83	
9/25	1511	1280	634	630	49.2	118	0.59	10,0	9,6	14.90	86	
10/2	2012	1517	665	846	55.7	133	0.68	9,8	14,0	15.45	98	

* Poikilocytosis of red cells of moderate degree. Experimental history, table 4–b.

Table 6 illustrates several points. This dog had been under observation for some time under a variety of dietary conditions (see experimental history—table 6-b). A period of sugar feeding gave no blood regeneration (refer to table 16 in the following paper) and 2 weeks of meat feeding had caused a considerable rise in the pigment volume.

The increase in pigment volume brought this figure back to normal in 4 weeks of mixed diet. The blood volume figures are based on determinations made with dry oxalate. This procedure, as has been pointed out, causes a shrinkage of cells and a dilution of the dye which gives blood volume figures abnormally high. Note that the blood volume figures per kilo are 100 to 136 cc. This fact may account for some of the fluctuations in the estimated plasma volume.

TABLE 6

Blood regeneration—mixed diet (following metabolism experiment). Dog 17-28. Bull dog, female, adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/19	1620	1500	600	900	60	108	0.76	7,1	7,4	11.60	129	
	Diet: Sugar diet, January 19 to February 23. Lean meat diet, February 23 to March 10. Mixed diet, March 10 to May 7. (Refer to table 16)											
3/16	1010	1246	735	511	41	81	0.64	6,3	7,2	10.40	119	Good condition
3/23	973	1158	660	498	43	84	0.64	6,5	10,6	9.90	116	
4/13	1910	1645	757	888	54	116	0.71	8,2	22,0	12.30	134	
4/20	1385	1260	630	630	50	110	0.66	8,3	9,8	12.60	100	
5/7	1895	1709	769	940	55	111	0.66	8,4	13,2	12.50	136	Good condition

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Table 7 is placed in this paper to give a comparison between the mixed diet experiments and fasting periods. It will be noted that during this fasting period the blood regeneration is very slight. This point will be discussed at length in the next paper.

DISCUSSION

From a review of the tables it is at once obvious that the *pigment volume* and total *red cell volume* after hemorrhage are below the expected values. There are several obscure factors in this reaction which call

TABLE 6-B

Experimental history. Dog 17-28

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 9/13/16	Bread and milk			8.4	No blood volume data given
Bled 450 cc.				8.4	
End 10/25/16				8.8	Hb. and R.B.C. back to normal
Begin 1/19/17	Sugar and metabolism	1620	129	11.6	Table 16
Bled 750 cc.		549	87	10.4	Lean beef diet 2 weeks followed by mixed diet. Table 6
End 2/23/17		541	117	8.0	
Begin 5/7/17	Sugar + R.B.C., metabolism	1895	136	12.5	Table 76
Bled 854 cc.		594	76	12.0	
End 6/18/17		1085	154	8.3	Put on bread and milk diet
Begin 9/11/17	Sugar 2 weeks	1650	113	14.0	
Bled 794 cc.	Sugar 2 weeks + diamino-acid of gelatin	751	84	13.5	
End 10/19/17		1040	106	10.0	Beef heart 3 weeks, mixed diet 3 weeks
Begin 6/3/18	Desiccated kidney, bread and milk	1739	73	17.65	
Bled 949 cc.		792	62	16.50	(3 bleedings)
End 6/28/18		1300	74	16.10	
Begin 8/9/18	Sugar and Hb. intraperitoneally	2417	93	17.00	Table 80
Bled 1076 cc.		756	66	15.90	(3 bleedings)
End 11/13/18		2000	106	15.05	
Begin 12/2/18	Lean meat and gelatin	2118	105	15.70	Table 51
Bled 1140 cc.		708	82	15.05	(3 bleedings)
End 1/22/19		1380	99	14.65	
Begin 5/1/19	Fasting	2018	97	15.90	Table 14
Bled 980 cc.		763	71	14.75	(3 bleedings)
End 5/21/19		1007	86	11.50	Found dead. Renal calculi

for discussion. Let us take a type experiment with simple values. A dog with a hemoglobin of 100 per cent, hematocrit of 50 per cent and blood volume of 600 cc. will have a pigment volume of 600. On two successive days the dog is bled one-fourth of the estimated blood volume. Allowing for replacement of plasma which is known to take place, we should expect after these bleedings a hemoglobin of 55 per cent and total volume of red cells 165 cc. The plasma is constant under such conditions and will be as normal, 300 cc. volume. The blood volume therefore is 465 cc. and pigment volume 256 (465 × 0.55) which is 43 per cent of the pigment volume at the beginning of the experiment. If there is no reserve red cell influx we should expect a red cell volume of 55 per cent and a pigment volume of 43 per cent normal following two bleedings of one-fourth the total blood volume. The tables

TABLE 7

Blood regeneration—fasting. Dog 17-27. White bull mongrel, female, adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/11	2100	1500	735	766	51	140	0.99	7,1	10,4	15.8	95	
3/13	1627	1122	561	561	50	145	1.03	7,0	8,0	15.7	72	*
3/13	Diet: Bread and milk											
3/13	Bled 375 cc. No distress											
3/14	Bled 375 cc. No distress											
3/16	1005	1116	770	346	31	90	1.05	4,3	12,4	15.5	72	*
3/16	Fasting begun											
3/22	1165	1153	726	427	37	101	0.90	5,6	6,8	13.8	76	*
3/29	1086	1075	656	419	39	101	0.67	7,5	6,2	12.3	88	*
4/4	1213	1054	622	433	41	115	0.65	8,8	6,8	11.6	91	*
4/10	1240	1000	620	380	38	124	0.95	6,7	6,2	10,7	93	Excellent condition

* Slight anisocytosis.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes. Experimental history, table 15–b. See below.

show figures which are consistently below the expected values. This calls for a broad discussion of the various methods of blood volume determination and possible errors inherent in the various methods. We do not wish to review all these points at this time but hope soon to do so in connection with experiments dealing with the actual technique of blood volume determination.

Two possibilities are to be mentioned, however, in this discussion. First the blood volume values may be too high, calculated by the dye method, and the actual bleedings represent more than 50 per cent of the total blood volume. Second, the possibility that the body has the power to modify the ratio of cells to plasma in different parts of the body under normal or abnormal conditions. It is easy to mention these two possibilities but very difficult to adduce experiments which are conclusive. For example in the abnormal condition of shock it seems clear that there is a remarkable disturbance in the ratio of cells to plasma in different parts of the body.

It may not be wise to pursue this question further but best merely to call the reader's attention to the fact that there is a discrepancy between the expected and actual values for red cell volume and pigment volume after unit hemorrhages. This point will be taken up again. The *pigment volume* figures, however, give a clear-cut index of the curve of blood regeneration.

SUMMARY

The term *pigment volume* used in these papers is equivalent to blood volume times per cent hemoglobin. This means the volume of available red cell pigment circulating in the body at the time of blood volume determination.

Given a uniform degree of anemia we may observe the curve of red blood cell regeneration as influenced by a variety of diet factors. Anemia is produced by bleeding the dog one-fourth of the determined blood volume on each of two successive days.

Under these experimental conditions a diet of mixed table scraps will effect complete blood regeneration to normal in a period of 4 to 7 weeks.

Under similar experimental conditions there will be little blood regeneration during a fasting period—mainly a maintenance factor equivalent to the normal daily wastage of red cells.

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BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA

II. Fasting Compared with Sugar Feeding

Analysis of "Sparing Action of Carbohydrates"

G. H. WHIPPLE, C. W. HOOPER and F. S. ROBSCHEIT

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

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That there is a distinct difference between the blood regeneration during *fasting periods* as compared with *sugar diet periods* comes out from an analysis of the experiments given below (table 25-a). This difference in blood regeneration is not great but is distinctly in favor of the fasting condition,—in other words, *a dog will form more red cells and hemoglobin during a fasting period than during a similar period of sugar feeding.* In neither case is any nitrogenous material taken into the body, so whatever hemoglobin and red cell stroma may be formed must be constructed in the body from body protein or protein split products. The well known "sparing action of carbohydrates" must be considered in the analysis of these experiments given below. It appears that these experiments can be explained most satisfactorily on the basis of a certain protection or sparing of body protein on the part of the sugar, associated with a definite amount of conservation of protein split products.

Having established the curve of blood regeneration which is the result of a mixed diet subsequent to the anemia period, we wish to present experiments to show the type reaction associated with fasting or sugar feeding. In making any analysis of results it is necessary to know how much reserve capacity the normal dog possesses—how much regeneration of red cells or hemoglobin can be effected during periods of fasting or sugar feeding. We must take into consideration too the daily wear and tear of the red cells which is not an accurately established factor. We do not know the life history of the red cell in the normal or anemic dog. We do know the duration of life of the red cell in the normal human being (1), but this life cycle may be different in disease. The dog's red cells are extremely fragile and this may or may not indicate a shorter life cycle for dog than the established 30-day period for normal human beings. Data on this point are very much to be desired but in their absence we must postulate an unknown factor of red cell or hemoglobin replacement which is present in all our tables.

Any diet, therefore, which is capable of giving a rising curve of blood regeneration following simple anemia is doing two things. The diet is responsible for a replacement of the red cells (3 to 5 per cent per day) which are worn out day by day, as well as the rise in general level of red cell volume above the anemia level. For the sake of analyis we may assume that the replacement value for human beings and dogs is the same—about 3 per cent per day or complete replacement of the total volume of red cells in approximately 30 days. Under most favorable conditions we may see the volume of red cells regenerate from an anemia level of one-third or one-half normal back to 100 per cent within 4 or 5 weeks. To supply a deficit of 50 to 65 per cent of its red cell volume the body requires 30 days or more over and above its maintenance of red cell wastage. This wastage (wear and tear of red cells) in human beings may be 100 per cent in 30 days. This indicates the importance of this replacement factor and further emphasizes the fact that our curves show the reaction of the body *in excess* of this wastage or replacement value.

When we note a falling curve of hemoglobin and red cells after a long diet period we need not hastily postulate hemolysis or blood destruction from some hypothetical toxin. It may be safer to consider the possibility that the body can form no more hemoglobin or red cells to repair the daily wastage. Even the replacement fraction is not being supplied and the curve subsides gradually depending upon the life cycle of the red cells remaining.

EXPERIMENTAL OBSERVATIONS

The experimental procedures have been described in detail in the preceding communication. The majority of these dogs have been under observation in the laboratory since birth and we have studied their reaction to simple anemia following hemorrhage as influenced by a variety of diets. Some of these observations precede and others follow these tabulated fasting or sugar feeding experiments. The experimental histories given with each animal give a review of the many experiments done on the same dog. The value of comparison under these conditions is greater than obtains in a series of isolated experiments. The dogs were all very fat and well nourished at the beginning of these experiments and were able to tolerate the fasting or sugar periods without disturbance of health or activity.

During the metabolism experiments the dogs were kept in standard metabolism cages constructed with sharp pitch of the cage bottom to insure rapid and complete drainage of urine. The dogs were catheterized every 24 hours and the catheterized specimen, bladder washings and cage urine were made up to a uniform volume. A mixed specimen was preserved and duplicate Kjeldahl analyses made. A few cubic centimeters of acetic acid in the cage collection bottle insured an acid reaction in the urine. With care a male or female dog may be catheterized daily without causing any cystitis. After the catheterization the water or sugar solution was given by stomach tube.

TABLE 8

Blood regeneration—fasting—metabolism—splenectomy. Dog 17-37. White bull mongrel, female, age 10 months

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
3/26	927	850	459	391	46	109	0.80	6,8	9,4	10.60	80
3/26	Diet: Bread and milk										
3/28	Bled 212 cc.										
3/29	Bled 212 cc.										
3/30	313	626	451	175	28	50	0.68	3,7	15,4	10.40	60
3/30	Fasting begun										
4/2	403	651	475	176	27	62	0.74	4,2	6,4	9.50	70
4/9	492	769	500	269	35	64	0.64	5,0	7,2	8.50	90
4/16	532	729	474	255	35	73	0.66	5,5	5,0	7.70	96
4/23	540	772	502	270	35	70	0.66	5,3	7,8	6.90	112

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Fasting experiments. The two preceding experiments (tables 8 and 9) are to be compared as the experiments were done at the same time.

TABLE 8-A

Total urinary nitrogen—fasting. Dog 17-37

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
March 31	2.80	650	21.5	0 feces
April 1	2.30	405	21.1	Trace of feces
2	2.58	433	20.8	0 feces
3	3.19	345	20.6	0 feces
4	2.97	395	20.3	0 feces
5	3.56	418	19.9	Slight diarrhea
6	2.83	460	19.6	0 feces
7	2.88		19.3	0 feces
8	2.49	427	19.0	0 feces
9	2.80	385	18.8	0 feces
10	2.91	473	18.3	0 feces
11	2.69	359	18.3	0 feces
12	2.91	423	17.9	Trace of feces
13	2.60	371	17.6	0 feces
14	2.60	429	17.5	0 feces
15	2.85	411	17.3	0 feces
16	2.86	414	17.1	0 feces
17	2.60	447	16.8	0 feces
18	2.77	458	16.5	0 feces
19	3.19	396	16.3	0 feces
20	3.02	421	16.1	Trace of feces
21	3.42	416	15.8	0 feces
22	3.56	445	15.7	0 feces
23	3.86	471	15.1	0 feces

Dog given 400 cc. of water daily by stomach tube.

TABLE 8-B

Experimenal history—dog 17-37—splenectomy

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 12/15/16	Sugar + gelatin	630	66	10.5	
Bled 350 cc.				10.2	
End 1/11/17		544	84	8.1	Maximum regeneration 2 weeks, then lean meat diet 3 weeks. Mixed diet
Begin 3/26/17	Fastingmetabolism	927	80	10.6	Table 8
Bled 424 cc.		313	60	10.4	Table 73
End 4/23/17		540	112	6.9	Bread, milk, Blaud's pills, 11 weeks. Slight regeneration

Splenectomy 10/23/16.

The dogs are of the same litter but one had been splenectomized. We can make out no difference in the reaction of the splenectomized dog as compared with the control under these experimental conditions. During the fasting period there is a steady rise in hemoglobin, red cell hematocrit, red cell count, total red cell volume and pigment volume. The rise is most pronounced during the first week, as a rule. It is noted that the plasma volume remains constant or decreases slightly. This fact accounts for the increase in blood per kilo, due to loss of body weight.

TABLE 9

Blood regeneration—fasting—metabolism. Dog 17–38. White bull mongrel, female, age 10 months

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
3/26	1395	1090	490	600	55	128	0.80	8,0	15,8	9.8	111
3/26	Diet: Bread and milk										
3/28	Bled 272 cc.										
3/29	Bled 272 cc.										
3/30	443	738	531	207	28	60	0.73	4,1	12,2	9.3	79
3/30	Fasting begun										
4/2	532	819	573	246	30	65	0.74	4,4	10,6	8.6	95
4/9	491	723	441	282	39	68	0.65	5,2	9,6	7.5	96
4/16	585	770	439	331	43	76	0.62	6,1	12,0	6.8	113
4/20	585	750	420	330	44	78	0.51	7,6	11,4	6.3	119

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
Experimental history, see table 20–b.

The nitrogen figures for total urine are of interest. The daily output is fairly constant until we reach the last 4 days of the experiment which show a distinct increase above normal. It is possible that this increase represents the body protein disintegration which may be observed after a long period of fasting, giving rise to the premortal rise in nitrogen. Both these dogs recovered the lost weight promptly when placed upon a bread and milk diet. There is no evidence for an increase in body protein katabolism to supply the essentials for hemoglobin construction. The same remarks apply to the following experiment (table 10).

The above experiment (table 10) shows a considerable increase in red cells from 5,200,000 to 7,000,000 with an unchanged red cell hematocrit. Poikilocytosis is marked during this period and there is evidence for a certain amount of fragmentation of the red cells in this and other experiments of similar nature. In some instances there is evidence for a faulty construction of red cells during periods of stress when red cell regeneration is being accomplished with difficulty on a limited diet. It will be possible with the accumulation of much data to ascertain which diets favor stroma construction and which diet factors accelerate hemoglobin construction. It is evident at present that these two fac-

TABLE 9–A

Total urinary nitrogen—fasting. Dog 17–38

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
March 31	3.42	720	19.4	0 feces
April 1	2.16	412	19.1	0 feces
2	2.18	471	18.8	Trace of feces. Vomited
3	2.77	236	18.6	Moderate feces
4	2.74		18.1	0 feces
5	2.94	430	17.9	0 feces
6	2.86	442	17.7	0 feces
7	3.25	428	17.3	Slight diarrhea
8	2.86	422	17.1	0 feces
9	2.66	432	16.7	0 feces
10	2.97	403	16.3	Trace of feces
11	2.46	413	16.2	0 feces
12	2.83	434	15.9	Slight diarrhea
13	2.72	383	15.7	0 feces
14	2.72	443	15.5	0 feces
15	3.05	446	15.1	0 feces
16	3.58	418	14.9	0 feces
17	3.42	408	14.7	0 feces
18	3.25	441	14.4	0 feces
19	4.14	441	14.1	0 feces
20	4.09	726	13.8	0 feces
21	4.17	517	14.3	Slight diarrhea

Dog given 400 cc. water daily by stomach tube.

tors do not always run parallel under experimental conditions, as well as in disease.

TABLE 10

Blood regeneration—fasting—metabolism. Dog 16-160. Bull mongrel, female, age 2 years

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cen*	*per cent*				*kgm.*	*cc.*	
5/3		1241	633	590	47.6					10.45	119	
5/3	Diet: Bread and milk											
5/6					45.2	90				10.00		
5/20	1316	1125	523	591	52.5	117	0.70	8,3	14,2	10.60	106	R.B.C.fragment++
5/21	Bled 281 cc.											
5/22	Bled 281 cc.											
5/24	482	752	492	250	33.2	64	0.55	5,8	14,8	10.20	74	
5/25	Fasting begun. Metabolism											
5/31	585	688	455	226	32.8	85	0.82	5,2	6,0	9.10	76	* Poik.
6/5	591	672	437	225	33.5	88	0.72	6,1	6,8	8.40	80	* Poik.++
6/12	636	707	457	243	34.3	90	0.64	7,0	8,2	7.55	94	* Poik.++
6/12	Diet: 200 grams bread and 300 cc. milk. Metabolism discontinued											
6/18	696	791	510	274	34.6	88	0.88	5,0	5,4	8.15	97	* Poik.++
6/26	451	550	360	184	33.5	82	0.68	6,0	9,0	8.20	67	* Slight poik.
6/26	Diet: Changed to mixed diet											

* Poikilocytosis of red cells.

Experimental history, see table 18–b.

This second group of experiments (tables 11, 12 and 13) presents several factors in common. During the fasting period the blood regeneration was notable in two experiments during the first week following the bleeding. In fact this level was scarcely increased during the subsequent fasting period. This rapid increase during the first week may be explained by some reserve factor which is called in during the emergency period. The following weeks show little increase in pigment volume because the body can only supply the material needed to replace the daily wear and tear on the red cells. The third experiment (table 12) shows a more gradual rise in the pigment volume, hematocrit and hemoglobin during the entire fasting period.

TABLE 10–A

Total urinary nitrogen—fasting. Dog 16–160

DATE, 1918	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
May 26	1.76	270	21.8	Some water vomited
27	1.90	120	21.4	About 50 cc. vomited; 0 feces
28	1.85	145	20.8	No vomiting. 0 feces; dog very active
29	2.07	113	20.6	No vomiting. 0 feces
30	2.02	155	20.3	0 feces
31	2.10	188	20.1	0 feces
June 1	2.02	199	19.8	0 feces
2	1.96	134	19.3	0 feces. 300 cc. water
3	2.44	167	19.1	0 feces. Good condition
4	2.16	206	18.9	0 feces
5	2.41	180	18.5	Solid feces
6	2.13	207	18.3	Little feces
7	2.02	221	17.9	0 feces
8	1.79	191	17.6	0 feces
9	1.90	186	17.4	0 feces
10	2.02	181	17.3	0 feces
11	1.90	178	17.0	0 feces
12	2.30	190	16.6	Feces + in urine. Dog in excellent condition
13	1.73	225	16.4	Soft feces

Dog given 200 cc. water daily by stomach tube.

All three experiments show a rapidly developing "dietary deficiency disease" which develops after a period of bread and milk feeding subsequent to the fasting period. This dietary deficiency condition is characterized by ulcerated mucous membranes and much gastro-intestinal disturbance. We are inclined to the opinion that this condition is analogous to scurvy in human beings. In the near future we hope to report a series of experiments bearing upon this point, making clear the factors concerned in the development of this abnormal condition as well as its cure and prevention in the dog.

TABLE 11

Blood regeneration—fasting. Dog 18–103. Brindle bull, female, age 1 year

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per ecnt*	*per cent*				*kgm.*	*cc.*	
8/28	1540	1115	552	547	49.0	138	0.97	7,1	16,0	15.45	72	
8/28	Diet: Crackermeal and milk											
8/28	Bled 279 cc.											
8/29	Bled 279 cc.											
8/31	810	965	650	311	32.2	85				14.95	65	
8/31	Bled 241 cc.											
9/3	590	955	708	242	25.3	62	0.94	3,3	26,0	14.35	65	* Poik.
9/3	Fasting begun											
9/10	946	1076	692	358	33.3	88	0.72	6,1	13,0	13.15	82	
9/16	852	882	552	321	36.4	97	0.88	5,5	11,4	12.15	73	
9/25	745	774	484	275	35.5	96	0.69	7,0	7,2	10.90	71	
9/30	700	784	485	295	37.7	89	0.57	7,8	13,2	10.30	76	Good condition
9/31	Diet: 250 grams crackermeal, 300 cc. milk											
10/11	699	889	572	311	35.0	79				11.30	79	
10/18	692	822	544	274	33.3	84	0.74	5,7	4,8	10.25	76	* Poik.+
10/18	Diet: Mixed diet											
10/22	Death from dietary deficiency disease											

* Poikilocytosis of red cells.

No previous anemia experiments on this dog.

TABLE 12

Blood regeneration—fasting (followed by rice, bread and milk, then yeast). Dog 18-114. White bull, female, adult

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/1	2550	1830	698	1115	60.9	139	0.75	9,3	12,6	13.95	131	
5/1	Diet: Bread and milk											
5/2	Bled 458 cc.											
5/3	Bled 458 cc. No distress											
5/5	620	1000	690	301	30.1	62	0.86	3,6	14,0	12.60	79	
5/6	Fasting begun											
5/14	890	1043	619	394	37.8	85	0.76	5,6	8,0	11.30	92	* Poik.
5/21	899	968	598	375	38.7	93	0.86	5,4	6,0	10.45	93	* Poik.
5/28	1103	1077	588	478	44.4	102	0.69	7,4	6,4	9.55	108	
5/28	Diet: 200 grams bread, 300 grams rice, 500 cc. milk											
6/4	1166	1166	654	502	43.1	100	0.62	8,1	6,6	10.45	115	* Poik.++
6/5	Diet: 30 grams compressed yeast, 100 grams bread, 500 cc. milk											
6/11	1070	1084	609	464	42.8	99	0.56	8,9	7,4	10.35	105	* Poik++
6/18	1232	1109	572	526	47.4	111	0.57	9,8	6,0	10.30	107	* Poik.†
6/25	1167	1094	547	537	49.1	107	0.54	10,5	10,4	10.25	107	
7/1	1148	1125	594	516	45.9	102	0.65	7,8	11,6	10.00	112‡	
7/1	Diet: Changed to mixed diet. Extra food. Recovery—4 days											

* Poikilocytosis of red cells.

† June 16: Mucous colitis. No yeast given; milk boiled.
June 17: No mucus; 10 grams yeast given; milk boiled.
June 18: No mucus; 20 grams yeast given; 300 cc. fresh milk.

‡ Beginning ulceration of mucous membrane of mouth. Beginning salivation.

Autopsy (see table 14)

Dog 17-28. White bull, female. See experimental history, table 6-b.

May 22, 1919. Found dead and cold. Blood clotted. Left ventricle slightly hypertrophied. Lungs and pleurae negative. Slight hypostatic congestion in right lower lobe. Spleen soft and flabby; normal size; pale pink-gray and cellular. Peritoneal cavity is normal. No pigmentation and no adhesions. Gastro-intestinal tract not opened, but superficially not abnormal. Liver normal size and color; indefinite hazy patches in which lobules look washed out (0.5 to 2 cm. in diameter). Bladder contains a little syrupy pus-like urine; slight cystitis. Left kidney large and hard; few cysts on surface; large stone fills pelvis (2 x 10 cm. ±). Right kidney shrunken to small size (2 x 3 x 5 cm.); stone in pelvis. Marrow of femur almost all fat. Marrow of rib is normal. Pancreas is small and warty looking—chronic change involves all of lower arm and head, all but distal fourth of upper arm. No evidence of acute process. Ovaries negative. Uterus shows sites of placental attachment. Urine: obtained from bladder at post-mortem. Small amount, mostly pus. No sugar. Few hyaline casts. Many pus cells and small round epithelial cells, few partially destroyed red cells. Bacteria abundant.

TABLE 12–B

Experimental history. Dog 18–114

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 4/24/18	Cooked liver,	553	94	7.85	Slight anemia
Bled 279 cc.	bread, and	435	105	7.65	Table 63
End 5/29/18	milk	791	84	9.60	Maximum regeneration 2 weeks. Mixed diet
Begin 8/14/18	Powdered liver, cracker-	1773	106	12.20	
Bled 816 cc.	meal and	770	95	11.75	(3 bleedings)
End 9/27/18	milk	2190	112	12.90	
Begin 11/14/18	Cooked liver	1640	105	13.50	Table 61
Bled 976 cc.	only	592	81	12.50	(3 bleedings)
End 12/11/18		1902	111	13.65	Complete regeneration 3 weeks
Begin 5/1/19	Fasting	2550	131	13.95	Table 12
Bled 916 cc.		620	79	12.60	
End 5/28/19		1103	108	9.55	

The experiment given in table 14 is complicated by nephritis, pyelitis and bilateral renal calculi. The blood regeneration during the two weeks preceding death was normal in every way. If anything, the blood pigment production was above the average. This is of some interest because of the frequent occurrence of anemia in human beings associated with advanced chronic nephritis. This dog's death was

TABLE 13

Blood regeneration—fasting (followed by rice and bread and yeast vitamine). Dog 18–116. White bull, female, adult

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/1	2620	1930	852	1050	54.4	133	0.85	7,8	8,0	18.75	103	
5/1	Diet: Bread and milk											
5/2	Bled 483 cc.											
5/3	Bled 483 cc. No distress											
5/5	778	1245	884	355	28.5	62	0.79	3,9	12,8	17.45	71	
5/6	Fasting begun											
5/14	1178	1357	818	525	38.7	87	0.84	5,2	14,0	15.95	85	* Poik.
5/21	1025	1205	742	434	36.0	85	0.75	5,7	9,4	14.85	81	* Poik.++
5/28	1176	1233	720	519	40.7	95	0.73	6,5	7,6	13.85	89	* Poik.++
5/28	Diet: 300 grams rice, 200 grams bread											
6/4	1109	1265	798	462	36.5	88	0.62	7,1	9,8	14.75	86	* Poik.++
6/5	Diet: 1 gram yeast vitamine,† 100 grams bread, 500 cc. milk											
6/11	1065	1232	761	458	37.2	86	0.70	6,1	8,2	14.65	84	* Poik.+
6/18	1368	1323	730	580	43.8	103	0.54	9,6	10,2	14.60	91	* Poik.+
6/25	1280	1380	780	594	43.0	93	0.51	9,2	5,6	14.05	98	* Poik.++
6/25	Diet: Changed to mixed diet. Developed dietary deficiency disease											
6/30	Death											

* Poikilocytosis of red cells.

† Vitamine prepared according to Seidell's method (2).

certainly due to renal injury and insufficiency, but the picture was not that of a primary essential chronic nephritis. In this single instance the development of a subacute renal disease did not impair the function of the organs of the body which are responsible for blood and hemoglobin regeneration.

Sugar feeding experiments. The first three experiments are similar and give strong evidence to show that there is very little regeneration of red cells and hemoglobin during a sugar feeding period. The reaction is constantly in favor of the fasting period during which time the dog can make a definite gain in red cells and hemoglobin, in excess of the maintenance requirements. In the first two experiments (tables 15 and 16) we have control observations in the same dogs during fasting periods (tables 7 and 14). The regeneration is distinctly more during fasting. In all three experiments the gain in pigment volume is present in the first week and we may wish to assume some emergency reserve to account for this reaction. The following weeks show little

TABLE 13-B

Expermiental history. Dog 18–116

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 4/24/18	Meat extract,	903	99	9.75	Table 65
Bled 540 cc.	bread and	422	89	9.65	(3 bleedings)
End 5/29/18	milk	865	89	10.85	Mixed diet 2 weeks. Complete regeneration
Begin 8/14/18	Thyroid,	1842	95	14.20	
Bled 944 cc.	crackermeal	1055	98	14.25	(3 bleedings)
End 10/24/18	and milk	1640	114	11.85	Complete regeneration 7 weeks
Begin 12/2/18	Beef heart	2120	115	14.90	Table 52
Bled 1160 cc.	and liver	776	90	13.95	(3 bleedings)
End 12/30/18		2270	106	15.50	
Begin 5/1/19	Fasting	2620	103	18.75	Table 13
Bled 966 cc.		778	71	17.45	
End 5-28-19		1176	89	13.85	

or no subsequent gain and may even show a falling off which indicates that the body cannot fabricate sufficient hemoglobin and red cells for its daily needs.

Splenectomy (table 17) done some time before this experiment does not appear to modify the reaction of the red cells and hemoglobin under the conditions of these experiments.

TABLE 14

Blood regeneration—fasting—nephritis and renal calculi. Dog 17-28. White bull, female, adult

DATE 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.	
5/1	2018	1550	706	829	53.5	130	0.76	8,6	7,0	15.9	97	
5/2	Diet: Bread and milk											
5/2	Bled 390 cc.											
5/3	Bled 390 cc. No distress											
5/5	763	1040	686	349	33.5	73	1.14	3,2	9,4	14.75	71	
5/6	Bled 200 cc. No distress											
5/8	631	956	648	295	30.8	66	0.94	3,5	8,2	14.25	67	
5/8	Fasting begun											
5/14	903	1041	643	415	39.9	87	0.79	5,5	7,8	13.0	80	
5/21	1007	988	535	432	43.8	102	0.74	6,9	8,2	11.5	86	*
5/22	Found dead. Autopsy given below											

* Dog is sick, weak and thirsty; distended abdomen.
Experimental history, see table 6-b.

The three metabolism tables (tables 15-a, 16-a and 17-a) in general show the characteristic reaction in urinary nitrogen. In every instance at the beginning when the anemia reaction is most intense we note the expected drop in urinary nitrogen when sugar is administered. The "sparing action of carbohydrate" is not disturbed by the presence of this degree of secondary anemia. The first and second experiments show a distinct rise in urinary nitrogen associated with the bleeding periods. These dogs showed considerable distress during the second bleeding period and it is highly probable that more than one-fourth of the total blood volume was removed on these two days (note the high figure, 130 cc. per kgm., for blood volume as determined by the dry oxalate method). We are inclined to attribute this rise in urinary nitrogen to the shock of the bleeding—to the tissue injury produced by the hemorrhage, and this observation is in harmony with those of Buell (3) working with pigs. Our third experiment (table 17-a) shows little or no increase in urinary nitrogen, no clinical reaction and a low estimated blood volume (80 cc. per kgm.).

TABLE 15

Blood regeneration—sugar feeding—metabolism. Dog 17-27. White bull mongrel, female, adult

DATE 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/19	1630	1507	603	904	60.0	108	0.71	7,6	9,0	11.60	130	Fasting
1/20	Bled 375 cc.											
1/22	Bled 375 cc.											
1/24	598	950	627	323	34.0	63	0.73	4,3	8,2	10.50	91	
1/24	Diet: 50 grams cane sugar, 25 grams glucose											
2/2	806	1203	698	506	42.0	67	0.56	6,0	6,0	9.6	125	* Anis.++
2/9	763	1090	643	447	41.0	70	0.57	6,1	8,8	9.0	121	* Anis.++
2/14	750	1028	596	432	42.0	73	0.70	5,2	7,6	8.2	125	Dog refuses sugar

* Anisocytosis of red cells

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Compare fasting period, table 7.

After the first exhibition of sugar we note a uniform low level of urinary nitrogen excretion. This low level is constant until the end of the experiments except in the first (table 15-a). Here we note a rise in urinary nitrogen during the last five days. It is fair to say that this

TABLE 15-A

Total urinary nitrogen—sugar. Dog 17-27

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
January 17	3.25	467	25.9	Fasting
18	2.58	411	26.0	
19	2.58	461	25.6	
20	2.86	422	25.1	Bled 375 cc.
21	3.75	381	24.1	0 feces
22	4.09	330	24.0	Bled 375 cc. Very weak
January 23	Anemia period begun—Diet: 50 grams sugar, 25 grams glucose			
23	3.30	537	23.8	0 feces
24	3.39	511	23.1	Diarrhea
25	1.99	427	22.8	0 feces
26	1.74	367	22.6	0 feces
27	2.02	390	22.3	Slight diarrhea
28	2.69	430	22.2	0 feces
29	2.38	435	21.9	0 feces
30	2.63	404	21.9	0 feces
31	1.74	401	21.7	0 feces
February 1	1.62	417	21.6	0 feces
2	1.46	401	21.2	0 feces
3	1.62	459	20.9	0 feces
4	1.96	375	0.8	Feces+
5	1.76	377	20.6	0 feces
6	2.07	400	20.5	0 feces
7	lost		20.4	0 feces
8	1.60	520	20.2	0 feces
9	1.90	475	19.8	Diarrhea
10	2.35	482	19.3	Diarrhea
11	3.86	720	18.4	0 feces. Vomitus +. Dog is sick
12	4.76	568	17.7	Vomitus +. Refuses sugar solution
13	4.76	361	18.1	0 feces
14	3.67	541	18.0	0 feces
15	4.20	505	17.8	Diarrhea +. Milk diet

Dog given 400 cc. water daily by stomach tube.

dog was sick, vomited the sugar solution and had diarrhea. This period is properly considered as an interval of intoxication in which little sugar was retained and perhaps this amount favored the intestinal irritation which was conspicuous.

TABLE 15-B

Experimental history. Dog 17-27

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 9/13/16	Lean meat			8.3	
Bled 450 cc.				8.9	(3 bleedings)
End 10/24/16				11.4	Complete regeneration of Hb. and R.B.C.
Begin 1/19/17	Sugar, metabolism	1628	130	11.6	Table 15
Bled 350 cc.		598	91	10.5	
End 2/16/17		646	119	8.6	Bread and milk 5 months. Slight regeneration
Begin 10/17/17	Sugar, metabolism	2150	123	13.4	
Bled 826 cc.	Sugar, gelatin, metabolism	718	84	13.2	
End 11/24/17		915	108	10.1	Sugar, gelatin, crackermeal, lard and butter, 3 weeks. Dietary deficiency disease. Recovery 2 weeks
Begin 3/13/18	Fasting	1626	72	15.7	Table 7
Bled 750 cc.		1004	72	15.5	
End 4/10/18		1240	93	10.7	
Begin 6/3/18	Cooked thymus, bread and milk	1939	87	16.35	
Bled 1022 cc.		723	70	15.75	(3 bleedings)
End 6/28/18		1245	78	15.70	
Begin 8/9/18	Hemoglobin intravenously. Sugar	1914	90	16.15	Table 79
Bled 988 cc.		817	67	15.45	(3 bleedings)
End 8/30/18		1155	80	13.75	Killed September 3

Tables 18 and 18-b deserve special mention as they show the results of several experiments done under controlled conditions on the same dog. The experimental history refers to table 10, which shows the blood regeneration on this dog during a fasting period. There is a distinct increase in pigment volume, hematocrit and hemoglobin during the fasting period. We see in table 18 the same dog under identical conditions on a sugar diet. During the same interval we note practically the same value for pigment volume, hematocrit and hemoglobin at the beginning and end of the sugar feeding. There is a trifling rise during the first week but this is subsequently lost with return to the initial level. One of the tables to follow (table 21) shows the same dog on a sugar and gliadin diet which holds the pigment volume curve about on a level—no gain nor loss.

TABLE 18

Blood regeneration—sugar feeding—metabolism. Dog 17–28. White bull, female, adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/19	1620	1500	600	900	60.0	108	0.76	7,1	7,4	11.60	129	Fasting
1/20	Bled 375 cc.											
1/22	Bled 270 cc.											
1/23	Bled 105 cc.											
1/24	588	900	603	306	34.0	61	0.80	3,8	7,8	10.40	87	
1/24	Diet: 50 grams cane sugar, 25 grams glucose, 400 cc. water											
2/2	717	1121	684	437	39.0	64	0.62	5,2	7,2	9.40	120	* Anis.
2/9	636	1027	637	390	38.0	62	0.65	4,8	6,2	8.90	115	
2/16	634	961	586	375	39.0	66	0.59	5,6	10,0	8.50	113	Diarrhea +
2/23	541	933	562	373	40.0	58	0.56	5,2	9,0	8.00	117	

* Anisocytosis of red cells.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Experimental history, see table 6–b.

Table 19 is unsatisfactory from the standpoint of the blood volume and pigment volume figures but in view of the other experiments we venture to include it because this dog illustrates a reaction which is not uncommon in dogs bled for the first time. In dogs used for the first time in anemia experiments we often note a remarkable regeneration of

TABLE 16–A

Total urinary nitrogen—sugar. Dog 17–28

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
January 17	2.46	482	25.9	Fasting
18	2.10	340	25.8	
19	2.46	425	25.6	Diarrhea
20	2.21	519	24.8	Bled 373 cc.
21	2.80	375	23.6	
22	3.08	320	23.6	Bled 270 cc. Dyspnoea
23	2.49	493	23.6	Bled 105 cc.
January 24	Anemia period begun. Diet: 50 grams sugar, 25 grams glucose			
24	2.46	495	22.8	
25	1.96	470	22.4	0 feces
26	1.99	432	22.3	0 feces
27	2.86	427	22.1	0 feces
28	2.69	440	21.8	0 feces
29	2.07	446	21.5	Diarrhea +
30	2.04	406	21.3	0 feces
31	1.71	398	21.2	0 feces
February 1	1.62	391	20.9	
2	1.79	396	20.6	Trace feces
3	1.60	452	20.4	0 feces
4	1.74	407	20.3	0 feces
5	1.68	395	20.1	0 feces
6	1.23	383	20.0	0 feces
7			19.9	0 feces
8	1.74	425	19.7	Feces +
9	1.48	398	19.6	
10	1.51	415	19.4	0 feces
11	1.43	356	19.3	0 feces
12	1.40	387	19.2	0 feces
13	1.48	394	19.1	0 feces
14	1.51	388	18.9	0 feces
15	1.40	391	18.8	0 feces
16	1.26	390	18.6	Diarrhea +
17	1.51	378	18.4	0 feces
18	1.15	390	18.3	0 feces
19	1.23	399	18.1	0 feces
20	1.40	385	18.1	0 feces
21	1.43	409	17.9	0 feces
22	1.20	384	17.8	0 feces
23	1.12	346	17.6	0 feces
24	1.32	374	17.5	

Dog given 400 cc. water daily by stomach tube.

red cells and hemoglobin on unfavorable diets. This was not understood at first but it may be stated as a fact to be explained or not. It is possible that this type of dog has a greater reserve stored in its body from which it can construct red cells and hemoglobin on demand but a dog which has been bled at various times has not this large reserve. At any rate, this dog was able to give a remarkable exhibition of regeneration of red cells and hemoglobin during a short period of sugar feeding. The red cell hematocrit rose from 26 per cent to 47 per cent and hemoglobin from 62 to 121 per cent. This regeneration was almost completed during 4 weeks on a diet which is least favorable to blood regeneration.

TABLE 17

Blood regeneration—sugar feeding—metabolism—splenectomy. Dog 17-34. White bull mongrel, female, adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/19	886	883	459	424	48.0	103	0.69	7,3	14,8	10.9	81	Fasting
1/20	Bled 221 cc.											
1/22	Bled 221 cc.											
1/24	414	780	546	234	30.0	53	0.80	3,3	11,4	10.1	77	
1/24	Diet: 50 grams cane sugar, 25 grams glucose, 400 cc. water											
2/2	528	979	676	303	31.0	54	0.60	4,5	6,4	9.3	105	
2/9	536	893	607	286	32.0	60	0.62	4,8	6,2	8.8	101	* Anis.
2/16	497	888	604	284	32.0	56	0.57	4,9	7,8	8.3	107	
2/23	556	868	567	304	35.0	64	0.60	5,3	6,4	7.8	113	

* Anisocytosis of red cells.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Tables 20, 20-a and 20-b present data on sugar feeding which support the other experiments given above. This dog was observed during a fasting period (table 9) as well as during this sugar period and subsequently during other diet periods of hemoglobin regeneration. This experiment shows only a 2-week period of pure sugar feeding during

TABLE 17-A

Total urinary nitrogen—sugar. Dog 17-34

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
January 17	3.42	303	25.0	Fasting
18	3.30	424	24.4	Diarrhea +
19	2.63	406	24.0	
20	2.52	412	23.6	0 feces. Bled 221 cc.
21	2.58	326	22.9	
22	2.86	316	22.8	Bled 221 cc.
January 23	Anemia period begun. Diet: 50 grams sugar, 25 grams glucose			
23	2.66	526	22.8	Trace soft feces
24	2.35	436	22.2	
25	1.99	371	22.0	Trace feces
26			21.8	0 feces
27	2.46	351	21.6	Slight diarrhea
28	2.69	431	21.3	0 feces
29	2.60	371	21.2	0 feces
30	2.77	397	21.0	Trace of feces
31	1.68	382	20.8	0 feces
February 1	1.54	396	20.6	0 feces
2	1.46	396	20.4	
3	1.74	381	20.2	0 feces
4	1.63	426	20.0	0 feces
5	1.54	341	20.4	0 feces
6	1.51	406	19.8	0 feces
7			19.6	Diarrhea +
8	1.51	420	19.6	0 feces
9	1.48	406	19.3	
10	1.34	386	19.0	0 feces
11	1.51	380	18.8	0 feces
12	1.34	361	18.9	0 feces
13	1.60	412	18.7	0 feces
14	1.40	401	18.4	Trace of feces
15	1.40	381	18.4	0 feces
16	1.40	386	18.3	0 feces
17	1.37	396	18.0	Trace of feces
18	1.46	376	17.8	Trace of feces
19	1.46	432	17.7	0 feces
20	1.48	367	17.7	Trace of feces
21	1.34	411	17.6	0 feces
22	1.48	391	17.4	0 feces
23	1.62	431	17.1	0 feces
24	1.74	384	17.13	Diarrhea. Boiled milk diet

Dog given 400 cc. water daily by stomach tube.

TABLE 17-B

Experimental history. Dog 17-34. Splenectomy

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 12/15/16	Meat and			10.9	No blood volume
Bled 390 cc.	bread only				data
End 1/11/17		972	85	11.3	Complete regenera-
				12.5	tion of Hb. and R. B. C.
Begin 1/20/17	Sugar.	910	81	10.9	Table 17
Bled 442 cc.	Metabolism	413	77	10.1	
End 2/23/17		556	113	7.8	Bread and milk diet 5 months
Begin 6/3/18	Powdered	1331	63	16.6	
Bled 807 cc.	liver, bread	664	62	15.7	(3 bleedings)
End 6/28/18	and milk	960	70	15.3	

Splenectomy 10/3/16.

TABLE 18

Blood regeneration—sugar feeding. Dog 16-160. Bull mongrel, female, age 2 years +

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/28	1835	1103	456	654	58.2	166	1.1	7,6	9,6	10.15	109	* Slight poik.
8/28	Diet: Crackermeal and milk											
8/28	Bled 276 cc.											
8/29	Bled 276 cc.											
8/31	648	771	524	243	31.5	84				9.55	81	
9/1	Bled 193 cc.											
9/3	448	696	513	177	25.5	64	0.8	4,0	15,4	9.5	73	* Poik.+
9/3	Diet: 75 grams sugar, 25 grams dextrose by stomach tube											
9/10	561	792	577	211	26.7	71	0.96	3,7	9,4	8.65	92	* Poik.++
9/16	488	703	500	192	27.3	69	0.78	4,4	7,8	8.0	88	* Poik.++
9/25	429	664	474	182	27.4	64	0.61	5,2	9,6	7.35	90	* Poik.++

* Poikilocytosis of red cells.

For continuation of experiment see table 80.

TABLE 18-B

Experimental history. Dog 16–160

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 9/26/16	Mixed			5.70	No blood volume data
Bled 440 cc.				6.10	(4 bleedings)
End 11/29/16				7.30	Complete regeneration of Hb. and R. B. C.
Begin 2/12/17	Bread, milk and Blaud's pills	938	127	7.70	Table 68
Bled 488 cc.		423	80	7.40	
End 7/16/17		456	97	5.90	Maximum regeneration 17 weeks
Begin 10/17/17	Sugar and gliadin	1278	122	8.90	Metabolism
Bled 542 cc.		599	95	8.90	Table 21
End 12/3/17		755	106	6.30	Crackermeal, gelatin, lard, butter, 2 weeks. Mixed diet 5 weeks. Complete regeneration
Begin 5/20/18	Fasting	1316	106	10.60	Metabolism
Bled 562 cc.		482	74	10.20	Table 10
End 6/12/18		636	94	7.55	Bread and milk 3 weeks
Begin 8/28/19	Sugar 3 weeks.	1835	109	10.15	Table 18
Bled 745 cc.	Sugar and Hb. intravenously 1 week	448	73	9.50	(3 bleedings)
End 10/16/19		586	95	7.80	Dried yeast and crackermeal, 2 weeks. Slight regeneration. Table 80
Begin 2/20/19	Sugar and carrot juice	1375	99	11.30	Table 22
Bled 781 cc.		548	81	10.10	(3 bleedings)
End 3/18/19	Dried yeast, bread and milk Beef liver, bread and milk	474	86	8.75	
Begin 8/8/19	Beet tops, bread and milk	1220	97	10.80	
Bled 526 cc.		600	66	13.15	
End 12/5/19	Spinach, bread and milk Compressed yeast, spinach, bread and milk	799	95	8.60	

which time is recorded a moderate increase in red cells and pigment volume. This increase is somewhat above the average reaction under similar conditions and if sugar feeding had been continued for 2 more weeks a drop in hemoglobin, pigment volume and red cell hematocrit was to be expected.

Histidine (1.5 gram per day) added to this diet actually prevented the expected fall and is responsible for a *definite gain* in the first week of histidine feeding. In view of the constancy of the sugar feeding reaction in the third week of blood regeneration we attach considerable importance to this reaction. We feel that histidine may be in part concerned in the complicated endogenous reaction which is responsible for the final elaboration of the complex protein hemoglobin.

TABLE 19

Blood regeneration—sugar feeding. Dog 19-28. Fox terrier, female, adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
9/18	1193	790	341	441	55.8	151	0.94	8,0	8,4	8.35	94	
9/18	Diet: Crackermeal and milk											
9/20	Bled 198 cc.											
9/21	Bled 178 cc. Dyspnoea											
9/23	316	509	370	137	26.9	62	0.80	3,9	22,6	7.85	65	
9/23	Diet: 50 grams sugar daily with 150 cc. water											
10/2	637	637	349	284	44.5	100	0.81	6,2	8,6	6.90	92	
10/11					47.2	107				6.10		
10/18	562	464	242	219	47.3	121	0.89	6,8	7,8	5.75	81	*

* Hemolysis in tubes containing blood and dye. Reading of color unsatisfactory.

No previous anemia experiments on this dog.

The last 2 weeks of histidine feeding show merely a level curve of hemoglobin and pigment volume indicating that the maintenance factor alone is being supplied. Under sugar feeding alone a slowly falling curve would be expected, so that the reaction as a whole is strongly in favor of the histidine feeding as being a contributory factor in the hemoglobin regeneration under these experimental conditions. The small amount of histidine given is significant when we consider the percentage content of histidine in casein (2.5 per cent). It will be noted in a subsequent paper that casein is not an efficient food for hemoglobin regeneration.

TABLE 20

Blood regeneration—sugar and histidine—metabolism. Dog 17-38. White bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
9/11	1342	1231	505	726	59.0	109	0.59	9,3	14,0	11.60	106
9/11	Diet: Bread and milk										
9/12 9/13	Bled 308 cc. Bled 308 cc.										
9/15	468	884	619	265	30.0	53	0.68	3,9	12,8	11.30	78
9/15	Diet: 50 grams cane sugar, 25 grams dextrose, 300 cc. water										
9/21	572	805	550	290	36.0	71	0.71	5,0	11,8	10.30	78
9/28	574	755	468	287	38.0	76	0.58	6,5	6,6	9.60	79
10/1	Diet: 50 grams cane sugar, 25 grams dextrose, 1½ gram histidine, 300 cc. water										
10/5	668	795	468	326	41.0	84	0.68	6,2	8,4	8.90	76
10/12	735	826	471	355	43.0	89	0.58	7,7	7,2	8.00	103
10/19	684	786	456	330	42.0	87	0.60	7,3	9,2	7.30	107

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

The total nitrogen elimination is uniform and sustained at the expected low level except during the last week of the experiment when a decided increase is noted. This type of intoxication is not infrequent after long sugar diet periods and we believe is of gastro-intestinal origin. It is a fact that the hemoglobin and pigment volume show no increase during this period of increased protein katabolism but rather

TABLE 20-A

Total urinary nitrogen—sugar and histidine. Dog 17–38

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
September 15	Diet: 50 grams cane sugar, 25 grams dextrose, 300 cc. water			
	grams	*cc.*	*pounds*	
16	2.52	415	23.9	Diarrhea. Vomited 55 cc.
17	1.93	370	23.7	Diarrhea. Vomited
18	1.46	401	23.4	Soft feces. Vomited 100 cc.
19	1.40	315	23.2	Vomited 50 cc.
20	1.34	240	23.1	0 feces
21	1.65	290	22.5	Diarrhea
22	1.23	285	22.5	0 feces
23	1.34	346	22.1	Slight diarrhea
24	1.34	247	21.8	0 feces
25	1.28	271	21.8	Slight diarrhea. Vomited
26	1.40	275	21.4	0 feces. Vomited 45 cc.
27	1.29	225	21.4	Soft feces. Vomited 25 cc.
28	1.20	245	21.2	Slight diarrhea
29	1.29	336	20.8	Soft feces
30	1.29	251	20.7	0 feces
October 1	Diet: 50 grams cane sugar, 25 grams dextrose, 1.5 gram histidine, 300 cc. water			
October 1	1.15	317	20.5	Feces +
2	1.23	290	20.5	0 feces
3	1.57	303	20.2	Slight diarrhea
4	1.34	264	19.9	0 feces. Vomited
5	1.65		19.6	
6	1.48	152	19.5	0 feces. Vomited 55 cc.
7	1.62	620	18.6	0 feces. Vomited
8	1.85	416	18.3	0 feces. Vomited
9	1.68	111	18.6	
10	2.63	401	18.1	0 feces
11	2.35	261	18.3	Vomited
12	3.25	453	17.6	0 feces. Vomited
13	3.08	341	17.6	0 feces
14	3.14	300	17.4	0 feces
15	2.86	415	17.1	0 feces
16	2.88	266	16.9	0 feces
17	2.58	329	16.8	0 feces
18	2.24	308	16.3	Moderate diarrhea
19	1.68	281	16.1	Diarrhea. Fair condition

TABLE 20-B

Experimental history. Dog 17–38

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 12/18/16	Sugar and gelatin			8.60	No blood volume data
Bled 600 cc.				8.20	(4 bleedings)
End 1/11/17		532	93	7.00	Slight regeneration of Hb. and R. B. C.
Begin 3/26/17	Fasting, metabolism	1395	111	9.80	Table 9
Bled 544 cc.		443	79	9.30	
End 4/20/17		585	119	6.30	Bread and milk 3 months
Begin 9/11/17	Sugar	1341	106	11.60	Table 20
Bled 616 cc.	Sugar and histidine	468	78	11.30	
End 10/19/17	Metabolism	684	107	7.30	Beef heart followed by mixed diet
Begin 6/3/18	Gelatin, bread and milk	1621	86	12.15	
Bled 753 cc.		723	76	11.25	(3 bleedings)
End 6/28/18		1071	85	10.70	Mixed diet
Begin 8/8/18	Cooked brain, crackermeal and milk	2060	109	11.65	
Bled 825 cc.		526	73	11.20	(3 bleedings) Pregnant
End 9/27/18		1100	84	11.95	Maximum regeneration 3 weeks
Begin 2/6/19	Bread (343 grams) milk (200 cc.)	1873	117	12.15	Table 26
Bled 710 cc.		520	77	11.10	
End 3/19/19		1092	95	11.35	Maximum regeneration 3 weeks
Begin 3/31/19	Bread (100 grams) milk (500 cc.)	1275	96	12.00	Table 26
Bled 580 cc.		499	78	11.40	
End 4/9/19		691	91	10.00	Maximum regeneration 4 weeks

a slight loss in red cell hematocrit and pigment volume. This will be shown to be true in other *abnormal conditions* in which increased protein katabolism and urinary nitrogen excretion are observed.

TABLE 21

Blood regeneration—sugar and gliadin—metabolism. Dog 16–160. White bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
10/17	1397	1083	509	627	53.0	118	0.74	8,0	8,2	8.90	122	
10/17	Diet: Bread and milk											
10/19	Bled 271 cc.											
10/20	Bled 271 cc.											
10/22	600	844	574	270	32.0	71	1.04	3,4	11,4	8.90	95	
10/22	Diet: 75 grams cane sugar, 25 grams glucose, 300 cc. water											
10/29	617	812	552	260	32.0	76	0.88	4,3	8,2	8.0	101	*
11/5	770	794	548	246	31.0	97	0.87	5,6	6,0	7.4	105	*
11/5	Diet: 75 grams cane sugar, 25 grams glucose, 20 grams gliadin, 300 cc. water											
11/12	762	838	536	302	36.0	91	0.78	5,8	3,6	7.10	118	*
11/18	764	813	496	317	39.0	94	0.82	5,7	7,2	6.70	121	*
11/26	780	777	466	311	40.0	103	0.76	6,6	4,4	6.40	106	*
12/3	755	770	485	285	37.0	98	0.71	6,9	3,6	6.30	106	*

* Poikilocytosis and anisocytosis of red cells.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
Gliadin prepared in the usual way by dilute alcohol extraction of wheat flour.
Experimental history, see table 18–b.

Tables 21 and 21-a give more data on sugar feeding periods. The hemoglobin regeneration is rather more marked than in the average experiment during a 2-week period. We note again a remarkable increase in red cells (2,000,000) and hemoglobin (26 per cent) with a stationary red cell hematocrit (32 per cent). If this observation had not been recorded in other experiments we might suspect an error.

TABLE 21-A

Total urinary nitrogen—sugar and gliadin. Dog 16-160

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
October 22	Diet: 75 grams cane sugar, 25 grams glucose, 300 cc. water daily			
	grams	*cc.*	*pounds*	
23	2.77	413	18.6	0 feces
24	2.02	267	18.3	Solid feces
25	1.74	232	18.2	0 feces
26	1.46	178	17.8	Trace of feces
27	1.48	101	17.8	0 feces
28	1.62	251	17.8	0 feces
29	1.74	261	17.6	0 feces
30	2.10	256	17.3	Diarrhea +
31	1.43	217	17.5	0 feces. Vomited
November 1	1.51	131	16.8	0 feces
2	1.57	241	16.8	0 feces
3	1.46	471	16.0	0 feces
4	1.79	151	16.3	0 feces
November 5	Diet: 75 grams cane sugar, 25 grams glucose, 20 grams gliadin, 300 cc. water daily			
5	1.71	301	16.2	Trace feces
6	3.16	241	16.1	0 feces
7	3.53	192	15.9	Feces +
8	3.47	186	15.9	Diarrhea +
9	3.25	173	15.8	Diarrhea +
10	3.58	311	15.5	Diarrhea ++
11	3.25	277	15.4	0 feces
12	3.33	322	15.6	0 feces
13	3.47	301	15.3	Solid feces
14	3.53	319	15.1	Diarrhea +
15	3.50	156	15.1	Trace of feces
16	3.30	132	15.0	0 feces
17	3.25	176	14.9	Soft feces
18	3.30	151	14.7	Trace of feces
19	3.36	192	14.8	Diarrhea +
20	3.25	161	14.6	0 feces
21	3.36	156	14.5	Soft feces
22	3.22	151	14.4	Solid feces
23	3.58	156	14.4	Soft feces
24	3.47	182	14.3	Soft feces
25	3.36	181	14.2	Soft feces
26	3.33	173	14.1	Diarrhea +
27	3.47	222	13.9	Soft feces
28	3.22	152	14.0	Trace of feces
29	3.36	181	13.9	0 feces
30	3.08	168	13.9	Soft feces
December 1	3.70	166	13.9	0 feces
2	3.28	181	13.8	Trace of feces
3	3.42	163	13.8	0 feces

TABLE 22

Blood regeneration—sugar plus carrot extract—yeast, bread and milk. Dog 16–160. White mongrel, female, adult

DATE, 1910	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.	
2/20	1375	1114	508	601	53.9	123	0.70	8,8	6,0	11.30	99	
2/20	Diet: Bread and milk											
2/21	Bled 280 cc.											
2/22	Bled 280 cc.											
2/24	690	884	473	307	34.7	78	0.85	4,6	19,2	10.45	85	
2/24	Diet: 100 grams sugar, 100 cc. carrot juice,† 150 cc. water											
2/27	548	816	569	242	29.7	67	0.90	3,7	12,8	10.10	81	
3/5	500	818	568	238	29.1	61	0.71	4,3	11,6	9.65	85	
3/12	444	776	555	206	26.5	57	0.73	3,9	5,2	9.15	85	* Poik.++
3/18	474	750	530	211	28.2	63	0.79	4,0	6,8	8.75	86	* Poik.++ Shadow cells
3/18	Diet: 200 grams bread, 500 cc. milk											
3/26	520	812	575	232	28.6	64	0.71	4,5	7,4	9.20	88	* Poik.++
3/26	Diet: 200 grams bread, 3 grams yeast, 300 cc. milk											
4/2	516	816	556	251	30.8	63	0.63	5,0	6,2	9.45	86	* Poik.++
4/8	433	761	542	211	27.7	57	0.54	5,3	9,4	9.35	81	* Poik.++
4/14	416	726	517	197	27.5	57	0.57	5,0	7,2	9.30	78	* Poik.
4/21	535	828	553	275	31.2	65	0.59	5,5	9,4	9.40	88	
4/21	Diet: 200 grams cooked beef liver, 200 grams bread, 300 cc. milk											
4/28	631	851	542	305	35.8	74	0.70	5,3	20,4	10.30	83	R. B. C. fairly normal
5/7	1087	1055	548	496	47.0	103	0.71	7,3	10,4	10.70	99	
5/12	1153	1082	556	517	47.6	107	0.69	7,8	16,2	10.85	100	

* Poikilocytosis of red cells.

† Carrot juice = water extract of cooked carrots filtered and concentrated to one-third of its original volume.

Experimental history, see table 18–b.

This shows the possible fluctuation in hemoglobin content and size of red corpuscles which may be observed with a constant hematocrit. Probably many factors enter into this reaction which will be taken up again. The clinical summary of this dog shows a variety of diet periods including a fasting period (table 18-a).

Gliadin (20 grams per day) added to the sugar diet causes no increase in hemoglobin but merely a uniform maintenance factor. There is still further increase in red cells with poikilocytosis and we believe the evidence favors some red cell fragmentation under these conditions. The urinary nitrogen shows a uniformly low level during the sugar period and the expected level during gliadin feeding (table 21-a).

Table 22 gives the results of a second sugar regeneration period on the same dog (16-160) used in the preceding experiment (table 21). It will be observed that this sugar regeneration period is not as favorable and there is actually a loss in pigment volume amounting to about 30 per cent during a period of 4 weeks. This experiment includes another factor (carrot juice) which is obviously inert under these experimental conditions. This point will come up again in subsequent papers dealing with pigment derivatives. We wish to point out an unusual condition on February 24 after the 2 bleeding days when a low plasma volume (473 cc.) is recorded against the normal during the entire experiment of 500 to 550 cc. When the plasma volume returns to normal we note a fall in hemoglobin and red cell hematocrit. It is probable that the reaction is the result of the shock of the hemorrhage which is usually adjusted during the resting day intervening between the second bleeding and the second blood volume determination.

The second period of bread and milk feeding is included to substantiate the plasma volume figures. This reaction will be discussed in detail in the next paper.

Table 23 is given at the end of the series because one important factor separates it from all the other experiments,—*a bile fistula.* This dog (15-22) has been under observation in this laboratory for several years and many reports on bile excretion include experiments on this animal (4). It is known that traces of bile can gain entrance into this dog's intestine but the general condition of the dog is perfect. The reaction to anemia under fasting conditions in this dog is of particular interest as the absorption of pigments from the intestine may be excluded. It has been suggested by Addis (5) that absorption of some pigment complex from the intestine is a part of the body conservation of pigment materials. This and other experiments give no support to this interesting suggestion.

Bile pigment figures in a fasting period are given for this same dog in another publication (6). The constant presence of *urobilin* in the fasting bile of this experiment is noted in table 23 and discussed below. Total urinary nitrogen figures are not given here but were obtained in this experiment and average 3.08 grams per 24 hours. This is an average figure for a normal dog of similar weight and activity. Evidently the bile fistula does not modify the total nitrogen figures in this experiment nor in many others.

TABLE 23

Blood regeneration—fasting—metabolism—bile fistula. Dog 15–22. Brindle bull, male, age 4 years +

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
3/26	2200	1833	770	1063	58.0	120	0.77	7,8	12,6	15.40	119
3/26	Diet: Bread and milk										
3/28	Bled 460 cc.										
3/29	Bled 460 cc.										
3/30	673	962	654	308	32.0	70	0.83	4,2	18,6	15.10	63
3/30	Fasting begun										
4/2	890	1186	747	439	37.0	75	0.66	5,7	28,2	14.00	85
4/9	874	1136	625	511	45.0	77	0.70	5,5	14,6	12.50	91
4/16	1122	1069	556	513	48.0	105	0.71	6,4	6,2	11.40	94
4/23	1133	1059	540	519	49.0	107	0.66	8,1	7,2	10.40	116

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
No previous anemia experiments on this dog.
Urobilin constantly present in bile after 1st day of fasting period.
Total nitrogen in urine—average 3.08 grams per 24 hours.

The curve of hemoglobin and pigment volume is quite remarkable. There is even more marked a new formation of red cells and hemoglobin than is noted in an average normal dog. We must not forget the occasional abnormal regeneration which is noted in a normal dog which has never been bled—a reserve being forthcoming which returns the hemoglobin almost to normal in 3 weeks of fasting. This bile fistula dog had never been bled in any large amounts before. But the reserve reaction in this dog is truly remarkable and at the end of the fasting period we note a normal red count, a hemoglobin and red cell hematocrit figure close to normal and a considerable increase in pigment volume. It is noted that the plasma volume shows a decrease during the fasting period which gives the reason for the relatively low pigment volume. At any rate the capacity of this bile fistula dog for hemoglobin regeneration is in no way impaired and *may* be greater than normal.

TABLE 24

Fasting summary

DOG NUMBER	AFTER BLEEDING			TWO WEEKS LATER			THREE WEEKS LATER			FOUR WEEKS LATER		
	Pigment volume	Hematocrit R. B. C.	Hemoglobin	Pigment volume	Hematocrit R. B. C.	Hemoglobin	Pigment volume	Hematocrit R. B. C.	Hemoglobin	Pigment volume	Hematocrit R. B. C.	Hemoglobin
15–22	673	32.0	70	874	45.0	77	1122	48.0	105	1133	49.0	107
18–103	590	25.3	62	852	36.4	97	745	35.5	96	700	37.7	89
18–114	620	30.1	62	899	38.7	93	1103	44.4	102			
18–116	778	28.5	62	1025	36.0	85	1176	40.7	95			
17–38	443	28.0	60	491	39.0	68	585	43.0	76	585	44.0	78
17–37	313	28.0	50	492	35.0	64	532	35.0	73	540	35.0	70
16–160	482	33.2	64	591	33.5	88	636	34.3	90			
17–28	631	30.8	66	1007	43.8	102						
17–27	1005	31.0	90	1086	39.0	101	1213	41.0	115	1240	38.0	124
Fasting average......	615	29.65	65	813	38.5	86	889	40.23	94	840	40.74	94
Sugar average, table 25..................	515	30.87	63	648	35.5	75	593	36.4	76	614	37.04	74

For example, we know that the bile fistula liver contains more pigment as demonstrated by the microscope. There may be stored away in the liver or other tissues more of the pigment complex from which the pigment reserve is derived—to be turned into finished hemoglobin on emergency demand.

The last three tables (tables 24, 25 and 25-a) give the summary figures of all the experiments in this paper and one fact stands out clearly in table 25-a. The *average blood regeneration* is distinctly greater during a fasting period of 2 weeks than during a similar sugar period of 2 weeks. The difference is even more striking at the end of 3 weeks when the average gain in pigment volume during fasting is 274, contrasted with 78 on sugar feeding. Hemoglobin figures show an average gain of 29 per cent on fasting contrasted with 13 per cent on sugar diet.

TABLE 25

Sugar diet summary

DOG NUMBER	AFTER BLEEDING			TWO WEEKS LATER			THREE WEEKS LATER			FOUR WEEKS LATER		
	Pigment volume	Hematocrit R. B. C.	Hemoglobin	Pigment volume	Hematocrit R. B. C.	Hemoglobin	Pigment volume	Hematocrit R. B. C.	Hemoglobin	Pigment volume	Hematocrit R. B. C.	Hemoglobin
17–28	588	34.0	61	636	38.0	62	634	39.0	66	541	40.0	58
17–34	414	30.0	53	536	32.0	60	497	32.0	56	556	35.0	64
17–38	468	30.0	53	574	38.0	76	668	41.0	84	735	43.0	89
16–160	600	32.0	71	770	31.0	97	762	36.0	91	764	39.0	94
16–160	690	34.7	78	500	29.1	61	444	26.5	57	474	28.2	63
19–28	316	26.9	62	916	47.2	107	562	47.3	121			
17–27	598	34.0	63	763	41.0	70	750	42.0	73			
16–160	448	25.5	64	488	27.3	69	429	27.4	64			
Sugar average........	515	30.87	63	648	35.5	75	593	36.4	76	614	37.04	74
Fasting average, table 24............	615	29.65	65	813	38.5	86	889	40.23	94	840	40.74	94

TABLE 25-A

Gains made above minimum level after bleeding

	DURING TWO WEEKS (TOTAL)			DURING THREE WEEKS (TOTAL)		
	Pigment volume	Hematocrit R. B. C.	Hemoglobin	Pigment volume	Hematocrit R. B. C.	Hemoglobin
Nine experiments, fasting average......	198	8.85	21	274	10.58	29
Eight experiments, sugar average.......	133	4.63	12	78	5.53	13

DISCUSSION

The term "sparing action of carbohydrates" is familiar to all workers in metabolism and means that carbohydrate feeding will decrease the excretion of total urinary nitrogen by man or animal as compared with the fasting excretion of nitrogen. It is therefore assumed that the administration of carbohydrate actually spares the body protein. There are two theories for this sparing action of the carbohydrates: *a*, That the sparing action is at the source—that is, the sugar prevents tissue katabolism or spares protein in tissue cells; *b*, That the sparing action is a conservation of split products which, with the aid of carbohydrate radicles, are reconstructed into a variety of protein complexes and used in various parts of the body. Both theories have able advocates, but it is fair to say that most of the experiments can be used by a skilful proponent to support either hypothesis. An admirable review of the work in this field has been published recently by Janney (7).

Some experiments by Davis and Whipple (8) concerning the regeneration of liver cells following a unit injury can be said to support convincingly the theory of conservation of protein end products. In these experiments there is little liver cell repair during fasting periods but a rapid repair during sugar diet periods. This indicates that the body can form many new liver cells when sugar is available but not during a fasting period. These new liver cells must be formed from body protein split products, and this type of conservation of nitrogen is due to sugar feeding and cannot be explained by any amount of protein sparing at the source.

The experiments, summarized in tables 24, 25 and 25-a, follow the normal regeneration of another type of body cell: the normal red blood cell which is constantly being used up and reformed or reconstructed day by day. This is an admirable cell for a study of cell regeneration as its main constituent (hemoglobin) is very complex and easily and accurately measured. The amount of hemoglobin circulating in the body can be measured with considerable accuracy and therefore its curve of regeneration can be established with reasonable precision. When we compare periods of regeneration of hemoglobin during fasting and during sugar diet periods we find a constant difference which comes out clearly in an average figure of many experiments (table 25-a). This table shows that the fasting dog can regenerate more red cells and hemoglobin than a dog on a sugar diet. This figure is over and above the maintenance supply of hemoglobin which is needed to keep the level uniform and furnish the hemoglobin used up by the daily wear and tear of the circulation in the body.

This actual reconstruction of new red cells and hemoglobin must come from the body protein or its split products as no nitrogen is being supplied to the body. Evidently the body conserves very carefully the substances which are suitable for the elaboration of hemoglobin. The pyrrol complex is a peculiar feature of the hemoglobin molecule which can scarcely be formed in the body and may be an important determining factor in its reconstruction. How may we explain the increase in hemoglobin during fasting periods in excess of the reaction on sugar feeding? This surely cannot be explained by increased synthetic capacity due to the presence of sugar or the conditions should be reversed. If we assume that sugar may have a certain sparing action at the source we are able to suggest a plausible explanation. Suppose the sugar feeding does protect body protein from katabolism and therefore lessens the amount of available protein split products, we are then able to explain the smaller amount of hemoglobin produced, *provided* we assume that under all circumstances of need or limited diet the body conserves *all* the available protein building stones which go to make up the hemoglobin molecule. This seems to us to be the best explanation of the observed facts, but this opinion may be modified by further work.

It may be objected that the "maintenance factor" of red cells may vary in fasting as compared with sugar periods. This factor is not to be determined at this time, but we have no reason to suppose that the daily wastage of red cells should be greater on sugar feeding than during fasting periods. This question must be left open for the present.

Granting the facts as outlined above we may say that we have good proof to explain the "sparing action of carbohydrates" as due to a conservation of protein split products which aid in new protein construction as observed in liver repair (8). But these anemia experiments may be best explained by a "sparing action of carbohydrate" which protects at the source the body protein from katabolism. If this work is correct we may assume that the carbohydrate in the diet may have a double "sparing action"—*to protect the body protein at its source and to aid materially in the conservation of protein split products*, which are recast into new body protein. That one or the other of these two reactions may be dominant under varying conditions may be granted as probable.

No discussion of any phase of pigment metabolism is complete without proper consideration of the pigment *output in the bile*. The bile pigment represents in part at least under certain conditions the end product of hemoglobin degradation in the body, but in addition under certain conditions this bile pigment may represent certain constructive activities of the liver (9). It is possible that a part of this bile pigment produced by the liver and not derived from the degradation of hemoglobin may be an excess of pigment substance available for hemoglobin *production* but not so used and later discarded by way of the bile. This reaction might only take place in the liver when there was a considerable surplus of pigment elements in the food or elsewhere. Such conditions might not obtain in the body when it was deprived of all protein intake (fasting or sugar diet).

The bile pigment output, therefore, must be reviewed briefly in this place. We are able to refer to observations of this nature (6) and state that there may or may not be differences in the bile pigment excretion during fasting periods as compared with sugar feeding periods in bile fistula dogs. Certain experiments appear to show greater bile pigment elimination during sugar periods than during fasting periods but other experiments show little or no difference in the bile pigment figures. These differences may represent individual variations in these bile fistula dogs and the available data are not sufficient to establish any difference in bile pigment excretion under these conditions. One thing is quite clear—these bile fistula dogs during fasting or sugar periods do excrete a measurable amount of bile pigments (average of 15 to 30 mgm. bile pigment per 6 hour daily collection). This pigment results from the degradation of hemoglobin in the body or the production of pigment complex from other substances in the liver—in other words, a distinct loss of pigment material from the body.

One other point must be mentioned in this connection. During fasting periods in bile fistula dogs we have noted the invariable appearance of *urobilin in the bile.* In our experience this is the only condition which is constantly associated with urobilin production in the dog's liver. At times the bile pigments may be almost completely replaced by the urobilin pigment and this introduces a serious error in our analysis of bile pigment. It is probable (if not certain) that this urobilin is derived from the bile pigment and its increase therefore will be associated with a corresponding decrease in bile pigments. But we have no accurate quantitative analytical method for urobilin, although we can estimate bile pigments there present by precipitation of the calcium pigment compound, filtration and analysis of the acid alcohol derivative. It seems probable to us that the urobilin appearing in the bile fistula dogs is derived at least in part from the bile pigments in the bile ducts, due to the activity of bacteria which we know are responsible in part at least for this reaction in the intestine. During fasting periods the flow of bile is very sluggish and this inferior drainage of bile gives a favorable opportunity for the bacteria to multiply. It can be stated that bacteria are numerous in all bile fistula tracts and may at times set up an inflammatory reaction in the bile passages which will cause trouble. Flushing out the bile passages by cholagogue action as a rule gives relief to this condition.

It has occurred to us that this observation may have some significance as regards urobilin in the urine in a variety of conditions. There is no conclusive proof that urobilin is ever absorbed from the intestine. We have here proof that urobilin may be formed in the liver. It would seem safe to assume that the *hepatic origin of urobilin* should be considered in any analysis of this complex question. When the possibility is suggested that *urobilin may be formed at times in the liver*, it is obvious how difficult it is to exclude this *possibility* in the clinical conditions associated with which we note urobilin in the urine.

SUMMARY

During *fasting periods* after unit hemorrhages the normal dog can regenerate measurable amounts of red cells and hemoglobin.

This regeneration of red cells and hemoglobin *includes* the daily wastage of these elements, or the maintenance factor of the blood. The curve of hemoglobin regeneration represents the production of hemoglobin *in excess* of this unknown maintenance factor.

Bile pigment excretion under fasting or sugar diet conditions may be considered as uniform. A bile fistula dog may regenerate hemoglobin and red cells with at least equal and perhaps greater speed than a normal dog. The constant presence of *urobilin* in the bile of the fasting bile fistula dog is recorded and discussed from the standpoint of *urobilinuria*. The hepatic origin of urobilin is suggested.

During *sugar diet periods* the regeneration of hemoglobin and red cells is *distinctly less than during fasting periods* (table 25-a).

We believe that this observation may be explained by a double "sparing action of carbohydrates"—both sparing at the source or protecting body protein from katabolism as well as effecting synthetically a distinct conservation of protein split products. This postulates a strict conservation by the body of certain protein fractions which may be recast into hemoglobin. The presence of carbohydrate may facilitate this reaction but the actual new formation of hemoglobin may depend in part upon the type and amount of amino acid groups available from normal protein katabolism.

Histidine given with sugar appears to cause a production of hemoglobin over the control level. This amino acid may be one of the important elements in this hemoglobin regeneration complex.

Gliadin in the amounts used does not modify the hemoglobin reaction.

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BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA

III. Influence of Bread and Milk, Crackermeal, Rice and Potato, Casein and Gliadin in Varying Amounts and Combinations

C. W. HOOPER, F. S. ROBSCHEIT and G. H. WHIPPLE

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

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Early in our work it became evident that bread and milk did not constitute a favorable diet for the rapid regeneration of blood. This diet is palatable to dogs and maintains them in good nutritional condition for many weeks. As a result we began to use this diet as a maintenance diet to which other factors could be added which did or did not modify the curve of blood regeneration to be expected from the bread and milk factors alone. Because in many experiments we use bread and milk as a part of the diet it is essential that we understand clearly the effect of this diet under a variety of conditions. We therefore submit many experiments tabulated below to establish the normal blood regeneration of the dog which is limited to varying amounts of dried white bread and skim milk.

We must mention in passing the dietary deficiency disease which develops in dogs kept for long periods on a strict bread and milk diet. This disease condition resembles scurvy in human beings and is rapidly fatal if not energetically treated by antiscorbutic measures. This condition will be reviewed in a subsequent publication.

It will be noted from the experiments here outlined that bread and milk alone when given in large amounts may return the blood picture to normal in six weeks or longer. But when given in moderate amounts (100 grams dried bread and 500 cc. skim milk) this diet will rarely permit of complete blood regeneration. On this diet the hemoglobin, pigment volume and red cell hematocrit may be kept at a permanently subnormal level following the unit hemorrhages used in our experiments to produce uncomplicated secondary anemia. The value of establishing this fact is obvious when it is found that some food materials added to this diet will profoundly modify this reaction expected from bread and milk alone. These observations will be reported in subsequent communications.

A few experiments with two of the important constituents of the bread and milk diet are included. Casein and gliadin in sufficient amounts are able to modify somewhat the reaction expected from sugar feeding alone. Some work with incomplete proteins and mixtures of amino acids has been completed but the evidence, so far, is not conclusively in favor of any single amino acid as being responsible for this peculiar reaction which depends in great measure upon the capacity of the body to construct hemoglobin.

Crackermeal and milk were used at one period during the war when white bread or in fact any kind of bread was not available for obvious reasons. This crackermeal was purchased on the open market during the war period and its constitution is not accurately known. We were able to ascertain with reasonable certainty that this crackermeal contained 70 to 80 per cent wheat flour, but a considerable percentage of barley and rice flour. Other grains may possibly have been concerned. The fact remains that this mixture of wheat flour and other grain flours did not modify in any manner the reaction established recently for commercial white bread which at present is made almost wholly from wheat flour.

Rice, potatoes and milk are used in one large series of experiments. The amount of blood regeneration on this diet closely parallels that observed on a bread and milk diet. This diet also includes one other substance sometimes used in bread,—potato or potato flour. Any one of these three diets is a favorable maintenance diet to which other factors may be added to determine the value of the unknown substance in its relation to blood regeneration.

EXPERIMENTAL OBSERVATIONS

Unless otherwise noted, the same technique is used in these experiments which has been described above (paper I). The food mixtures were all palatable and readily eaten unless note is made to the contrary. With few exceptions the dogs maintained their weight, general activity and health throughout the experiments.

Bread and milk diet. The first experiment (table 26) in this series illustrates many points which are established by the succeeding experiments given below. The dog presented a very high hemoglobin (130 per cent), high red count (10 million) and blood per kilo (127 to 117 cc.). Following the unit hemorrhages there is recorded as usual a volume of red cells (239 cc.) which is much below the calculated expected red cell volume (438 cc.). The low level in this experiment is more noticeable than in the average experiment. The plasma volume is promptly made up to normal after the bleedings and remains as usual relatively constant. The curve of regeneration is quite steep during the first 2 weeks of the bread and milk diet but thereafter remains at a uniform level. This statement applies to the pigment volume, red cell hematocrit, and hemoglobin, but the red cell count shows a slow increase toward normal in the last 3 weeks of the experiment.

In this first experiment the diet was abundant and sufficient to maintain the body weight and even to allow of slight increase. This point is of much importance, as will appear later. This and other similar experiments show that a *liberal bread and milk diet* sufficient to maintain or increase the body weight will cause a certain degree of hemoglobin regeneration over and above the daily maintenance hemoglobin factor. Blood regeneration may be rapid for a week or two and may even return the blood picture almost to normal in certain experiments.

The repeat experiment on this same dog (table 26) shows a lower initial hemoglobin and red count. The hemorrhages are less in amount but the anemia level for pigment volume is much the same as in the preceding anemia period. The diet now is not abundant and contains only 100 grams dried bread as compared with 343 grams in the first period of regeneration. This 100 grams bread diet is not sufficient to maintain the body weight at its normal level and there is a loss in weight of 1.4 kilos during the 5 weeks of blood regeneration. There is a striking difference in the amount of hemoglobin regeneration which shows only a trivial increase from week to week over the maintenance factor. In using the term *maintenance factor* we wish to indicate that unknown replacement fraction which represents the daily wastage of red cells used up in the body metabolism. There are experiments to indicate that this fraction may be 3 per cent per day in human beings, but there are no data to establish this important point for the dog.

It is obvious from these two anemia periods (table 26) that a dog will regenerate more hemoglobin on an abundant bread and milk diet than on a limited bread and milk diet. This applies particularly to the *bread* portion of the diet. The term "bread" as used in this

TABLE 26

Blood regeneration—bread and milk—repeat experiment. Dog 17-38. Bull mongrel, female, young adult

DATE, 1910	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/30	1910	1467	585	877	59.8	130				11.5	127	
2/6	1873	1418	600	812	57.2	132	0.66	10,0	9,0	12.15	117	
1/30	Diet: White bread and milk											
2/7	Bled 355 cc.											
2/8	Bled 355 cc.											
2/10	520	852	608	239	28.1	61	1.0	3,1	28,4	11.1	77	
2/10	Diet: Dried, ground white bread, 343 grams, skim milk, 500 cc.											
2/17	852	1074	654	404	37.6	79	0.88	4,5	12,0	11.6	93	
2/17	Diet: Dried, ground white bread, 343 grams, skim milk, 200 cc.											
2/26.	1120	1133	588	534	47.1	99	0.72	6,9	11,8	11.6	98	
3/3	1137	1110	560	544	49.0	102	0.82	6,2	7,4	11.35	98	* Poik. +
3/10	1138	1083	575	497	45.9	105	0.72	7,3	7,8	11.5	94	* Poik. +
3/19	1092	1072	561	493	46.0	102	0.66	7,7	6,8	11.35	95	* Poik. +
3/21	Mixed diet											
3/31	1275	1155	558	586	50.7	110	0.67	8,2	7,2	12.0	96	* Poik. ++
3/31	Diet: White bread and milk											
4/1	Bled 290 cc.											
4/2	Bled 290 cc. No distress											
4/3	499	887	633	249	28.1	56	0.67	4,2	9,6	11.4	78	* Poik. +
4/3	Diet: Dried, ground white bread, 100 grams, skim milk, 500 cc.											
4/11	566	989	662	307	31.0	64	0.68	4,7	11,0	11.25	77	*
4/18	662	988	638	335	33.9	67	0.56	6,0	9,2	11.0	90	*
4/25	652	920	578	304	35.4	71	0.55	6,5	6,6	10.7	86	*
5/2	734	955	585	367	38.4	77	0.57	6,7	7,2	10.35	92	* Poik. ++
5/9	691	909	564	341	37.5	76	0.58	6,5	5,6	10.0	91	* Poik. ++

* Poikilocytosis of red cells.

Experimental history, see table 20-b.

TABLE 27

Blood regeneration—bread and milk—repeat experiment. Dog 19–94. Bull mongrel, male, age 5 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/16	1250	1343	790	540	40.2	93	0.57	8.1	21,6	10.65	126	
1/16	Diet: Bread and milk											
1/17	Bled 335 cc.											
1/18	Bled 285 cc. No distress											
1/20	462	839	645	190	22.6	55	0.95	2,9	14,6	10.00	84	
1/20	Diet: 250 grams dried, ground white bread, 500 cc. skim milk											
1/27	576	1020	683	327	32.0	56	0.55	5,1	11,0	10.25	100	*
2/3	900	1088	651	485	39.7	83	0.57	7,3	13,4	10.25	106	* Poik. +
2/12	750	1000	626	362	36.2	75	0.72	5,2	10,2	10.80	93	* Poik. +
2/19	907	1085	647	439	40.4	84	0.69	6,1	9,8	11.15	97	*
2/28	994	1126	667	448	39.8	88	0.76	5,8	6,2	11.50	98	*
3/7	899	1098	650	437	39.8	82	0.73	5,6	8,4	11.70	94	*
3/10	Diet: Mixed diet											
3/17	1146	1216	687	523	43.0	94	0.70	6,7	10,6	13.05	93	* Slight
3/17	Diet: White bread and milk											
3/18	Bled 304 cc.											
3/19	Bled 304 cc. No distress											
3/21	542	968	709	255	26.3	56	0.82	3,4	14,4	12.40	78	
3/21	Diet: 300 grams dried, ground white bread, 500 cc. skim milk											
3/28	736	979	640	324	33.1	75	0.89	4,2	12,6	12.50	94	* Slight
4/2	811	1112	712	395	35.5	73	0.78	4,7	8,4	12.45	89	*
4/9	864	1110	680	420	37.8	78	0.65	5,9	11,4	12.40	89	* Slight
4/16	1005	1200	718	469	39.1	84	0.70	6,0	8,0	12.75	94	* Slight
4/23	1094	1236	740	485	39.2	88	0.71	6,9	6,0	12.75	97	* Slight
4/30	1031	1311	800	498	38.0	79	0.55	7,2	10,0	13.20	99	* Slight

* Poikilocytosis of red cells.

No previous anemia experiments with this dog.

report indicates white bread of the first quality, obtained from the University Hospital, sorted, dried in an oven and pulverized.

Table 27 gives a repeat experiment which shows that the curve of blood regeneration following a very short resting period is practically identical under uniform diet conditions. It is of considerable importance to know beyond question whether we may expect a uniform reaction under uniform conditions when a dog is used for different experiments at different intervals of time. We believe this communication includes sufficient data to establish this point beyond question. Therefore we may feel secure in using for anemia work the same set of dogs, *provided* the blood picture has returned to normal and the weight and general health is also normal. With repeated anemia experiments the dog does not increase in its capacity to regenerate hemoglobin nor does this reparative mechanism fail under the conditions of these repeat experiments. We may then attach considerable significance to deviations from the standard reaction in any given animal.

The repeat experiment (table 27) gives a reaction curve of pigment volume, red cell hematocrit and hemoglobin which is practically identical with the first anemia period. There is slightly more gain in the repeat experiment than in the first anemia observation. In both anemia periods the diet was liberal and permitted a gain in body weight of approximately 1 kilo per 6-week period. The repeat experiment brought the hemoglobin back more nearly to normal but the initial anemia level was not as low nor was the loss by hemorrhage as great. In the repeat experiment the dog received 300 grams dried white bread in contrast to 250 grams bread in the first period, but this was only a proper proportion per kilo body weight. These dogs, moreover, were in a period of rapid body growth (5 to 8 months).

The anemia experiments given in table 28 are very similar to those just described. In this instance, too, the bread and milk diet was sufficient for maintenance plus a definite growth factor with a gain of 2 kilos in body weight during the 5-week periods. In both periods the regeneration brings the pigment volume and blood picture back to the normal level. The steady gain in weight and body growth must not be lost sight of in reviewing the same gain in plasma volume. In the adult normal dog the plasma volume is now known to be quite constant under these experimental conditions.

The experiment given in table 29 is slightly different from those preceding. After the anemia period we have 1 week's fast which shows as usual a definite gain in pigment volume. The subsequent 3 weeks

TABLE 28

Blood regeneration—bread and milk—repeat experiment. Dog 19–102. Bull mongrel, male, age 5 to 6 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.	
1/30	972	944	482	453	48.0	103				9.75	97	
2/6	1026	1068	606	452	42.3	96	0.63	7,6	16,0	10.90	98	
2/6	Diet: Bread and milk											
2/7	Bled 267 cc.											
2/8	Bled 267 cc. No distress											
2/10	418	803	594	205	25.5	52	0.79	3,3	11,4	10.25	78	
2/10	Diet: 283 grams dried, ground white bread, 500 cc. skim milk											
2/17	720	951	608	337	35.5	76	0.93	4,1	8,6	10.65	89	
2/26	888	1014	592	417	41.1	87	0.65	6,7	11,6	11.25	90	* Poik. +
3/3	1045	1068	575	488	45.7	98	0.76	6,4	12,8	11.35	94	* Poik. +
3/10	1020	1045	578	462	44.2	97	0.73	6,6	11,2	11.55	91	* Poik. ++
3/19	1026	1056	576	467	44.2	97	0.70	6,9	12,0	12.55	86	* Poik. ++
3/22	Diet: Mixed diet											
3/31	1220	1220	644	564	46.2	100	0.67	7,5	12,6	13.20	93	* Poik. ++
3/31	Diet: Bread and milk											
4/1	Bled 305 cc.											
4/2	Bled 305 cc. No distress											
4/3	470	874	634	232	26.5	54	0.68	4,0	16,8	12.50	70	* Poik. ++
4/3	Diet: 283 grams dried, ground white bread, 500 cc. skim milk											
4/11	758	1085	702	361	33.3	70	0.69	5,1	7,4	13.30	82	*
4/18	884	1083	650	421	38.9	82	0.64	6,4	12,8	13.50	80	
4/25	1099	1163	660	498	42.8	94	0.64	7,3	9,6	13.85	84	*
5/2	1648	1485	701	769	51.8	111	0.74	7,5	11,2	14.55	102	*
5/9	1280	1268	667	584	46.0	101	0.64	7,9	19,8	14.50	87	*

* Poikilocytosis of red cells.

No previous anemia experiments with this dog.

show a stationary pigment volume up to the last week, when there is a definite gain. This diet was sufficient for maintenance of body weight and the general condition was uniformly excellent.

TABLE 29

Blood regeneration—bread and milk. Dog 18-123. Brindle bull mongrel, male, young adult

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/1	1692	1270	546	716	56.9	133	0.68	9,8	12,2	12.55	101	
5/1	Diet: Bread and milk											
5/2	Bled 318 cc.											
5/3	Bled 318 cc. No distress											
5/5	592	826	552	270	32.7	72	0.64	5,6	18,8	11.60	71	
5/6	Bled 200 cc.											
5/8	440	668	459	203	30.3	66	0.85	3,9	12,0	10.90	61	
5/8	Fasting											
5/14	620	802	515	283	35.3	77	0.73	5,3	12,2	10.30	78	
5/14	Diet: 100 grams dried, ground white bread, 500 cc. skim milk											
5/21	638	852	560	270	31.7	75	0.73	5,1	17,0	9.95	96	* Poik. +
5/28	633	891	582	299	33.6	71	0.66	5,4	14,2	9.90	90	* Poik. +
6/4	798	929	574	345	37.2	86	0.63	6,8	10,6	9.75	95	* Poik. +

* Poikilocytosis of red cells.

No previous anemia experiments on this dog.

The experiments given in tables 30 and 31 are very similar and may be discussed together. In both experiments the amount of bread and milk was not measured, but it is significant that there was a slight but definite loss of body weight during the bread and milk periods. It is to be expected, therefore that the diet would not suffice to raise the level of hemoglobin and pigment volume much above the anemia level. The larger dog (table 30) does show a slow regeneration toward normal in 4 weeks, but the smaller dog (table 31) shows almost no net gain during 3 weeks. There is a little gain in the first week which is lost subsequently.

The mixed diet reaction is very nicely shown in both experiments (tables 30 and 31) and a week or two is sufficient to make up the deficit in hemoglobin and establish the normal level.

TABLE 30

Blood regeneration—bread and milk. Dog 18-113. Bull mongrel, female, age 4 to 5 months

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
4/24	970	1000	561	426	42.6	97	0.63	7,7	9,6	9.75	103	
4/24	Diet: Bread and milk—amount not measured											
4/25	Bled 250 cc.											
4/26	Bled 170 cc. No distress											
4/29	418	853	559	277	32.5	49	0.52	4,7	12,6	9.50	90	
5/8	597	776	468	295	38.0	77	0.61	6,3	8,6	9.13	85	
5/15	878	1140	674	445	39.1	77	0.49	7,9	9,6	9.10	125	*Fragm.
5/22	666	912	555	341	37.3	73	0.52	7,0	10,2	9.30	98	*Fragm.
5/29	701	788	473	315	40.0	89	0.55	8,1	9,8	9.10	87	*Fragm.++
5/29	Diet: Mixed diet											
6/10	940	940	530	397	42.2	100	0.60	8,4	10,2	10.45	90	*Fragm.

* Fragmentation of red cells.

No previous anemia experiments on this dog.

A *bile fistula* experiment is included (table 32) to show that these dogs react like normal dogs as regards blood pigment production after simple anemia. This dog was known to have complete exclusion of bile from the intestinal tract (autopsy notes). His general condition during the entire experiment was excellent and the bread and milk diet was sufficient to maintain the body weight close to normal. During the entire period there was a loss of only 1.4 kilos, which is not great when we consider the size of the dog and the length of the experiment (7 weeks). There was a slight initial gain in hemoglobin and pigment volume which was subsequently lost. The general level of pigment volume, red cell hematocrit and hemoglobin is pretty nearly uniform with occasional temporary gains and losses. It is of interest to note a steady gain in number of red cells from 3,400,000 to 6,600,000. The hemoglobin gain was much less, which gives a fall in color index from 0.87 to 0.54. This is not uncommon in normal dogs under similar experimental conditions.

TABLE 31

Blood regeneration—bread and milk. Dog 18-115. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.	
4/24	655	780	394	368	47.2	84	0.59	7,1	14,2	7.00	111	
4/24	Diet: Bread and milk—amount not measured											
4/25	Bled 195 cc.											
4/26	Bled 140 cc.											
4/29	314	582	375	200	34.3	54	0.63	4,3	26,2	7.30	80	
5/8	572	752	467	274	36.45	76	0.59	6,5	9,4	6.77	111	
5/15	675	888	522	353	39.8	76	0.59	6,4	25,0	6.40	138	
5/22	468	593	354	233	39.3	79	0.70	5,6	6,0	6.20	96	
5/23	Diet: Mixed diet											
5/29	516	607	373	228	37.5	85	0.64	6,6	10,8	6.90	88	* Poik.
6/10	769	761	438	318	41.8	101	0.73	6,9	14,2	8.00	95	* Poik.

* Poikilocytosis of red cells.

No previous anemia experiments on this dog.

Crackermeal, milk, lard and butter. These crackermeal experiments were performed in part during the war period when white bread was not available. This crackermeal consisted of an unknown mixture including at least wheat, barley and rice flours, possibly others. The experiments show reactions which resemble accurately those observed with white bread. A mixture of grain flours, therefore, is no more efficient in promoting a regeneration of hemoglobin and red cells than white bread alone, consisting mainly of wheat flour.

Table 33 is to be compared with table 30—the first experiment done with white bread and milk, and the second with crackermeal and milk.

TABLE 32

Blood regeneration—bread and milk diet—bile fistula. Dog 17-151. White bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
5/28	2675	2158	971	1085	55	124	0.72	8,6	5,2	18.20	113
5/28	Diet: Bread and milk										
5/29	Bled 540 cc.										
5/30	Bled 540 cc.										
5/31	830	1408	1000	408	29	59	0.87	3,4	14,6	17.50	80
5/31	Diet: Bread and milk—amount not measured										
6/8	1058	1557	1059	498	32	68	0.77	4,4	9,8	17.20	90
6/15	1575	1852	1222	630	34	85	0.65	6,5	6,6	16.80	110
6/22	1274	1464	937	527	36	87	0.63	6,9	10,8	16.40	89
6/29	990	1415	920	495	35	70	0.49	7,2	7,4	16.10	88
7/6	1150	1532	950	582	38	75	0.54	6,9	5,8	15.90	96
7/13	1480	1741	1097	644	37	85	0.55	7,7	13,8	15.90	109
7/18	1118	1574	1039	545	34	71	0.54	6,6	13,8	16.10	98

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
No previous anemia experiments on this dog.

These experiments were performed during a period of rapid growth and the diets in each instance were sufficient to preserve body weight. There was a slight gain in weight during the crackermeal experiment. Both experiments show a slow steady gain in pigment volume, hemoglobin and red cell hematocrit. We may say the curves are as nearly identical as one can hope to observe in this type of experiment.

After a period of 8 weeks of crackermeal and milk diet the dog suddenly developed acute *dietary deficiency disease* which resulted in death 1 week later. Note the fall in blood volume, hemoglobin and red cell hematocrit during this short period. The autopsy findings will not be discussed at this time.

TABLE 33

Blood regeneration—crackermeal and milk. Dog 18–113. Bull mongrel, female, age 8 to 9 months

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/14	1556	1136	571	557	49.0	137	0.81	8,4	5,2	12.85	88	
8/15	Diet: Bread and milk											
8/16	Bled 284 cc.											
8/17	Bled 284 cc.											
8/19	714	978	699	270	27.6	73				12.45	79	
8/19	Bled 220 cc.											
8/21	804	1200	858	323	26.9	67	0.84	4,0	26,2	12.35	97	
8/21	Diet: 200 grams crackermeal, 500 cc. milk											
8/27	788	984	667	308	31.3	80	0.80	5,0	12,4	12.50	79	* Poik.
9/4	894	1048	670	367	35.0	85	0.66	6,4	8,6	12.55	84	* Poik. ++
9/11	1078	1135	688	441	38.9	95	0.65	7,3	6,4	12.55	90	* Poik. ++
9/19	1136	1152	700	442	38.4	99	0.67	7,4	18,0	12.60	92	* Poik. ++
9/27	1380	1315	737	558	42.5	105	0.67	7,8	14,8	12.90	102	* Poik. +
10/9	1085	1119	640	497	44.4	97	0.63	7,7	11,0	12.80	93	
10/17	1290	1277	728	536	42.0	101				12.75	100	
10/18	Diet: Mixed diet. Extra meat. Dietary deficiency disease											
10/25	788	1050	690	355	33.8	75	0.58	6,5	10,8	10.35	101	
10/26	Killed. Autopsy											

* Poikilocytosis of red cells.

Refer to table 30, bread and milk experiment.

Table 34 presents a long experiment in which the diet is crackermeal, lard and butter in sufficient amounts to preserve the body weight and permit of a gain of 0.7 kilo during the period of 12 weeks. No previous anemia experiments had been performed on this dog, which may account for the fact that the blood regeneration finally carried the level to normal. This reserve has been mentioned in the preceding communication. The gain is very slow and at times there appears to be a slight loss in red cell hematocrit or hemoglobin or pigment volume.

TABLE 34

Blood regeneration—crackermeal, lard and butter. Dog 17-205. Bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
10/29	1450	1058	571	487	46	137	0.87	7,9	8,6	10.70	99
10/29	Diet: Bread and milk										
10/30	Bled 265 cc.										
10/31	Bled 265 cc.										
11/2	522	790	563	229	29	66	1.14	2,9	27,0	10.40	76
11/2	Diet: 200 grams crackermeal, 10 grams lard, 10 grams butter										
11/9	559	860	585	275	32	65			6,2	10.30	83
11/16	948	1088	664	425	39	87	1.01	4,3	18,8	10.30	105
11/23	969	1052	589	463	44	92	0.90	5,1	9,2	10.30	102
11/28	998	998	549	449	45	100	0.78	6,4	9,2	10.30	97
12/5	1111	1028	555	473	46	108	0.86	6,3	8,8	10.40	99
12/10	1070	1008	544	464	46	106	0.82	6,5	13,6	10.20	99
12/19	904	913	511	404	44	99	0.77	6,4	13,6	10.60	86
12/26					44	119	0.79	7,5	8,0	10.60	
1/2/18					48	124	0.78	7,9	10,2	10.50	
1/9					46	127	0.79	8,0	12,8	10.70	
1/17					46	127	0.85	7,5	14,2	10.70	
1/23	1480	1139	592	546	48	130	0.69	9,4	13,4	11.10	103

No previous anemia experiments on this dog.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

The end result after 12 weeks of this diet may be accepted as normal. It is unusual that this dog tolerated this diet for such a prolonged period without any signs of dietary deficiency disease. A subsequent experiment (paper V) shows dietary deficiency symptoms in this same dog after a shorter period of a similar diet.

TABLE 35

Blood regeneration—crackermeal, lard and butter. Dog 16–160. White bull mongrel, female, age 12 months

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/4	1218	1006	533	473	47	121	0.67	9,1	7,2	10.00	100	
3/4	Diet: Crackermeal, lard and butter											
3/6	Bled 262 cc.											
3/7	Bled 242 cc.											
3/9	726	844	591	256	30	86	0.86	5,0	9,4	9.60	89	
3/9	Diet: 206 grams crackermeal, 10 grams lard, 10 grams butter											
3/15	732	842	581	261	31	87	0.72	6,0	8,4	9.30	91	
3/20	762	786	517	267	34	97	1.08	4,5	9,8	9.30	84	
3/27	840	785	526	259	33	107	0.89	6,0	8,6	9.20	85	Slight diarrhea
4/3	812	805	531	274	34	101	0.83	6,1	15,8	8.90	90	
4/9	845	836	535	301	36	101	0.78	6,5	12,6	9.10	92	

Experimental history, see table 18–b.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

The experiment given in table 35 is very much like the preceding one but for the fact that this dog had been observed previously in anemia periods (see experimental history 18-b). The blood regeneration is very slow on the same amount of crackermeal, lard and butter. There was a slight loss of weight on this diet which contains a sufficient number of calories per kilo. This is to be explained by the presence of diarrhea.

The addition of milk powder (table 36) to the lard, butter and crackermeal diet does not modify the curve of blood regeneration nor does it prevent the development of the dietary deficiency disease after a period of 1 month. The peculiar reaction of the red cell hematocrit which actually diminishes as the hemoglobin and red cell count increase may be explained in part by the use of dry oxalate in varying amounts.

TABLE 36

Blood regeneration—crackermeal, lard, butter and milk powder. Dog 16-140. Bull mongrel, male, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/4	2212	1616	711	905	56	137	0.77	8,9	6,8	15.10	107	
3/4	Diet: Crackermeal, lard and butter											
3/6	Bled 414 cc.											
3/7	Bled 394 cc.											
3/9	1027	1222	880	465	38	84	0.87	4,8	13,2	14.30	85	
3/9	Diet: 163 grams crackermeal, 10 grams lard, 10 grams butter, 100 ± grams milk powder											
3/15	1215	1322	846	476	36	92	0.88	5,2	11,2	14.8	89	
3/20	1153	1281	883	448	35	90	0.88	5,1	9,6	15.0	85	
3/27	1055	1227	834	392	32	86	0.84	5,1	8,0	14.80	83	
4/3	1350	1324	834	490	37	102	0.77	6,6	19,8	14.60	91	
4/9	1408	1257	855	402	32	112	0.84	6,7	11,6	14.10	89	*

* Dietary deficiency disease. Death April 17.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

The pigment volume regeneration is about the expected amount in view of the diet which was sufficient to maintain but not increase the body weight.

The next three experiments may be discussed in a group (tables 37, 38 and 39). The influence of splenectomy is concerned in two of these experiments and under these experimental conditions the blood regeneration appears to progress in a normal fashion, at least for a time.

TABLE 36-B

Experimental history. Dog 16–140

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 9/13/16	Mixed			8.1	
Bled 450 cc.				8.4	(3 bleedings)
End 10/13/16				10.1	
Begin 11/6/16	Fasting, metabolism			9.3	
Bled 660 cc.	Sugar, metabolism			8.2	(4 bleedings)
End 12/16/16	Gliadin, sugar			6.4	
	Gelatin, sugar				
Begin 9/11/17	Sugar, metabolism	1570	109	13.1	
Bled 712 cc.	Mono-amino-acid	756	90	12.6	
End 10/19/17	fraction of gelatin	1273	130	9.3	
Begin 3/4/18	Crackermeal, lard,	2210	107	15.1	Table 36
Bled 808 cc.	butter, milk powder	1025	85	14.3	
End 4/9/18		1385	89	14.1	Dietary deficiency disease

TABLE 37

Blood regeneration—crackermeal, lard, butter, alfalfa meal. Dog 18-97. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME*	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
3/11	2038	1358	584	774	57	150	0.88	8,5	9,2	12.40	109
3/11	Diet: Bread and milk										
3/13	Bled 339 cc.										
3/14	Bled 340 cc.										
3/16	908	966	570	396	41	94	1.04	4,5	15,6	11.40	82
3/16	Diet: Crackermeal, lard, butter, 40 grams alfalfa meal										
3/22	1175	1118	548	570	51	105	0.86	6,1	22,2	10.70	103
3/29	1250	1058	561	498	47	118	0.75	7,9	14,6	10.80	98
4/4	1322	1076	581	495	46	123	0.74	8,3	12,4	10.90	99
4/10	1598	1102	595	508	46	145	0.75	9,7	11,4	11.00	100

*Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
No previous anemia experiments on this dog.

TABLE 38

Blood regeneration—crackermeal, lard and butter—splenectomy. Dog 17-163. Bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
9/3	1110	1069	588	481	45	104	0.72	7,2	6,2	10.0	106	
9/3	Diet: Bread and milk											
9/4	Bled 279 cc.											
9/5	Bled 279 cc.											
9/7	388	719	532	187	26	54	0.90	3,0	22,4	9.70	74	
9/7	Diet: 200 grams crackermeal, 20 grams lard											
9/14	610	813	512	301	37	75	1.01	3,7	10,2	9.60	85	
9/21	728	867	512	355	41	84		5,8	5,6	9.60	91	
9/22	Diet: 200 grams crackermeal, 10 grams lard, 10 grams butter											
9/28	754	820	492	328	40	92	0.72	6,7	8,0	9.60	85	
10/5	704	800	456	344	43	88	0.73	6,0	13,4	9.80	80	
10/12	806	848	517	331	39	95	0.82	5,8	5,6	9.70	87	
10/17	886	914	539	375	41	97	0.88	5,5	6,6	9.70	94	
10/25	868	914	585	329	36	95	0.79	6,0	8,0	9.80	93	
10/31	145	596	518	78	13	24	0.75	1,6	20,4	9.70	61	*

* Death. (Internal hemorrhages, urobilin in urine ++.)
Blood volume by dry oxalate. Hemoglobin by Sahli tubes.

TABLE 38-B

Experimental history. Dog 17-163 (splenectomy)

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 4/24/17	Meat, Blaud's pills	918	102	9.0	Table 75
Bled 458 cc.		340	76	8.9	
End 6/6/17		1063	117	8.6	
Begin 9/3/17	Crackermeal, lard	1112	106	10.0	Table 38
Bled 558 cc.	Crackermeal, lard, butter	388	74	9.7	
9/21/17		728	91	9.6	
10/25/17		868	93	9.8	
End 10/31/17		143	61	9.7	Death 11/1/17

Table 38 illustrates a not infrequent condition which develops in splenectomized dogs made anemic and fed on a limited diet. We have pointed out elsewhere (1) that there is a remarkable condition which may develop in splenectomized bile fistula dogs. In these bile fistula dogs if anemia is produced we may observe periods of spontaneous blood destruction and enormous pigment overproduction. Under such conditions it was suggested that the body was forming its maximum amounts of pigment material (hemoglobin as well as bile pigment) but the red cell stroma was lacking in quantity or quality. It was suggested that the spleen might be concerned in the development of red cell stroma. These present exeriments may point to the production of faulty red cell stroma under such conditions (table 38) but some readers may wish to postulate the development of some unknown poison to explain the distintegration of the red cells in our splenectomy experiments. This condition develops very abruptly and may super-

TABLE 39

Blood regeneration—crackermeal, lard, butter, alfalfa meal—splenectomy. Dog 17-34. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
3/11	1776	1345	740	606	45	132	0.94	7,0	11,8	16.10	84
3/11	Diet: Bread and milk										
3/13	Bled 336 cc.										
3/14	Bled 336 cc.										
3/16	828	1034	765	269	26	80	1.14	3,5	22,0	15.30	68
3/16	Diet: Crackermeal, lard, butter, 50 grams alfalfa meal										
3/22	1128	1128	722	407	36	100	1.00	5,0	17,4	14.80	76
3/29	1282	1187	760	427	36	108	0.93	5,8	17,0	15.20	78
4/4	1185	1162	732	430	37	102	0.77	6,6	16,2	15.00	77
4/10	1371	1193	740	453	38	115	1.08	5,3	16,8	14.80	81

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
Experimental history, see table 17-b.

vene in a dog with relatively normal red cell count and hemoglobin values. Within 2 or 3 days the red cell count may fall to one-third normal (table 38) and abnormal pigments appear in blood serum and urine. Much more study must be given to this condition and we expect to report further work in this line.

Alfalfa meal was added to the diets in two of these experiments. These experiments give no evidence to indicate that alfalfa meal exerts a definite influence upon the curve of hemoglobin regeneration in the dog. The alfalfa meal used in our experiments was the usual grade of finely ground alfalfa purchased on the open market.

Rice, potatoes and milk. We may consider the next group of experiments as a unit (tables 40, 41, 42, 43 and 44). In principle all these experiments are similar and the results are remarkably uniform. In the first four experiments the dogs were bled and placed upon a uniform diet of cooked rice, boiled potatoes and skim milk. The regeneration in most of the experiments was slow but uniform with the end result after 5 to 6 weeks about normal or slightly below the normal blood level. After this there followed a short period (7 to 10 days) of mixed diet. Then a second period of anemia and blood regeneration upon the same rice, potato, milk diet was observed. These second periods are replicas of the first regeneration periods on this same diet.

It is clear that a liberal diet of cooked rice and potato with skim milk sufficient to maintain or slightly increase the body weight will give a slow steady gain in blood pigment, red cell hematocrit, red cell count, etc., which will often bring the regeneration curve back to normal or close to normal.

Two experiments are exceptions to the general reaction (tables 40 and 44). Table 40 shows a regeneration which is incomplete and not back to normal in 5 weeks. In fact, during the last month the regeneration is not in evidence and the pigment volume, hemoglobin and red cell hematocrit are stationary. There was a slight loss of weight during this period but the dog was very active and normal in all respects. The second anemia regeneration period shows an identical reaction. Table 44 shows a still more striking difference from the normal average regeneration. This dog refused to eat the amounts of rice and potato and milk given at first. She ate the amounts recorded in table 44, which amount to about 50 per cent that given to the other dogs, or 50 calories per kilo body weight. During 6 weeks there was a loss of 2.5 kilos and the blood regeneration was only slight during this whole period.

TABLE 40

Blood regeneration—rice, potatoes and milk—repeat experiment. Dog 19-104. Bull mongrel pup, male

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm*	*cc.*	
1/30	1367	1067	455	602	56.4	128				8.75	122	
2/6	1394	1078	468	594	55.1	129	0.68	9,5	14,8	9.00	120	
2/6	Diet: Bread and milk											
2/7	Bled 270 cc.											
2/8	Bled 270 cc. No distress											
2/10	363	630	457	170	26.9	58	0.76	3,8	25,8	8.35	75	
2/10	Diet: 363 grams rice, 417 grams potatoes, 500 cc. milk											
2/17	692	765	451	311	40.6	90	0.82	5,5	11,6	8.00	96	
2/26	933	859	451	404	47.0	109	0.75	7,3	8,4	8.00	107	
3/3	807	816	445	366	44.9	99	0.70	7,1	10,2	7.90	103	* Poik.+†
3/10	910	833	419	410	49.2	109	0.80	6,8	10,0	7.85	106	* Poik.
3/19	814	773	402	362	46.9	105	0.67	7,8	9,0	7.85	98	* Poik.+‡
3/21	Diet: Mixed diet. Extra food											
3/31	1036	898	433	456	50.8	115	0.69	8,3	12,2	8.65	104	
3/31	Diet: Bread and milk											
4/1	Bled 225 cc.											
4/2	Bled 225 cc. No distress											
4/3	462	688	462	223	32.4	67	0.88	3,8	24,8	8.20	84	
4/3	Diet: 363 grams rice, 417 grams potatoes, 500 cc. milk											
4/11	656	780	469	300	38.4	84	0.70	6,0	11,2	8.20	97	* Poik.
4/18	701	772	435	330	42.7	91	0.56	8,1	8,8	8.05	96	
4/25	760	764	421	339	44.4	98	0.55	8,9	8,0	8.00	95	
5/2	844	816	421	391	47.9	103	0.64	8,1	7,8	7.90	103	
5/9	791	804	431	369	45.9	98	0.58	8,5	5,8	7.65	105	

* Poikilocytosis of red cells.

† Only 300 cc. of milk given.

‡ Gave 300 grams rice and 300 grams potatoes.

No previous anemia experiments on this dog.

TABLE 41

Blood regeneration—rice, potatoes and milk—repeat experiment. Dog 19–95. Bull mongrel, male, age 5 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/16	1232	1130	595	528	46.4	109	0.67	8,2	18,0	11.35	100	
1/16	Diet: Crackermeal and milk											
1/17	Bled 282 cc.											
1/18	Bled 282 cc. No distress .											
1/20	560	824	602	218	26.4	68	0.77	4,4	30,4	11.00	75	
1/20	Diet: 418 grams boiled rice, 490 grams potatoes, 500 cc. milk											
1/27	742	974	574	395	40.6	76	0.72	5,3	16,2	12.75	76	
2/3	954	976	531	435	44.6	98	0.65	7,5	22,8	11.15	87	
2/12	982	1013	538	464	45.8	97	0.75	6,5	14,2	10.90	93	
2/19	1040	1000	511	480	48.0	104	0.70	7,4	10,6	10.60	95	
2/28	1242	1058	514	538	50.9	118	0.88	6,7	9,8	10.80	98	* Poik.+
3/7	983	1006	521	475	47.2	98	0.87	7,2	10,0	11.20	90	* Poik.+
3/10	Diet: Mixed diet											
3/17	1092	1130	640	484	42.8	97	0.84	5,8	13,0	12.80	88	* Poik.
3/17	Diet: Crackermeal and milk											
3/18	Bled 283 cc.											
3/19	Bled 283 cc. No distress											
3/21	570	934	664	251	26.9	61	0.92	3,3	18,0	12.45	75	* Poik.
3/21	Diet: 418 grams boiled rice, 490 grams potatoes, 500 cc. milk											
3/28	577	834	576	250	30.0	69	0.86	4,0	6,0	11.75	79	* Poik.
4/2	741	953	605	334	35.0	78	0.63	6,2	11,6	11.80	81	* Poik.+
4/9	773	976	600	371	38.0	79	0.70	5,6	8,2	11.85	82	
4/16	988	1046	588	448	42.8	94	0.69	6,8	10,4	11.90	98	
4/23	1085	1073	576	492	45.8	101	0.68	7,4	20,0	11.95	90	
4/30	1237	1168	576	581	49.7	106	0.65	8,1	8,2	11.95	98	

* Poikilocytosis of red cells.

No previous anemia experiments on this dog.

TABLE 42

Blood regeneration—rice, potatoes and milk—repeat experiment. Dog 19–93. Bull mongrel, female, age 5 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	*per cent*	*per cent*				*kgm.*	cc.	
1/16	1435	1380	742	616	44.7	104	0.59	8,9	18,6	10.95	126	
1/16	Diet: Bread and milk											
1/17	Bled 345 cc.											
1/18	Bled 245 cc. No distress											
1/20	594	886	640	237	26.8	67	0.76	4,4	15,2	10.25	86	
1/20	Diet: 400 grams boiled rice, 475 grams potatoes, 500 cc. milk											
1/27	768	1084	638	436	40.2	71	0.48	7,4	10,8	10.35	105	* Slight
2/3	1058	1080	570	494	45.7	98	0.60	8,1	15,2	9.55	113	* Slight Cells small
2/12	1040	1063	566	482	45.3	98	0.69	7,1	8.4	9.90	107	*
2/19	1245	1163	570	582	50.0	107	0.73	7,3	6,4	9.55	122	* Slight
2/28	1255	1125	568	540	48.0	112	0.84	6.7	5,8	9.65	116	* Slight
3/7	1098	1127	568	542	48.1	98	0.73	6,7	6,0	10.35	113	* Slight
3/10	Diet: Mixed diet											
3/17	1040	1004	516	478	47.6	104	0.70	7,4	7,8	11.35	89	* Slight
3/17	Diet: Bread and milk											
3/18	Bled 251 cc.											
3/19	Bled 251 cc. No distress											
3/21	566	916	647	260	28.4	62	0.84	3,7	12,2	11.15	82	*
3/21	Diet: 400 grams boiled rice, 475 grams potatoes, 500 cc. milk											
3/28	896	1028	614	397	38.6	87	0.85	5,1	7,8	11.35	90	
4/2	908	1085	631	433	39.9	84	0.65	6,5	8,6	11.20	97	* Slight
4/9	858	990	568	412	41.6	87	0.60	7,2	6,0	11.35	87	
4/16	1136	1112	565	530	47.7	102	0.67	7,6	8,8	11.30	98	* Slight
4/23	1212	1142	568	557	48.8	106	0.57	9,3	6,8	11.35	100	
4/30	1313	1198	572	604	50.4	110	0.58	9,5	7,4	11.15	107	* Slight

* Poikilocytosis of red cells.

No previous anemia experiments on this dog.

TABLE 43

Blood regeneration—rice, potatoes and milk—repeat experiment. Dog 19–103. Bull mongrel, female, age 5 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
1/30	1100	1077	588	483	44.9	102			·	9.55	113	
2/6	978	1020	598	428	41.9	96	0.61	7,9	12,6	10.00	102	
2/6	Diet: Bread and milk											
2/7	Bled 255 cc.											
2/8	Bled 255 cc. No distress											
2/10	430	808	600	204	25.3	53	0.78	3,4	9,0	9.45	85	
2/10	Diet: 411 grams rice, 472 grams potatoes, 500 cc. milk											
2/17	770	854	485	365	42.7	90	0.78	5,8	12,4	8.85	96	
2/26	996	981	520	446	45.5	102	0.68	7,5	10,8	9.10	108	
3/3	1305	1070	491	574	53.6	122	0.80	7,6	8,4	9.20	116	
3/10	1068	1008	526	476	47.3	106	0.68	7,8	7,0	9.20	110	
3/19	1065	986	499	477	48.4	108	0.72	7,5	9,0	9.60	103	*
3/21	Diet: Mixed diet. Extra food											
3/31	1103	1103	600	492	44.6	100	0.71	7,0	10,6	10.65	103	
3/31	Diet: Bread and milk											
4/1	Bled 276 cc.											
4/2	Bled 276 cc. No distress											
4/3	444	837	612	220	26.3	53	0.76	3,5	11,6	10.40	80	
4/3	Diet: 411 grams rice, 472 grams potatoes, 500 cc. milk											
4/11	756	964	603	347	36.0	79	0.76	5,2	8,8	10.70	90	
4/18	803	957	570	377	39.4	84	0.57	7,4	14,6	10.55	91	
4/25	970	1003	546	439	43.8	97	0.64	7,6	7,2	10.65	94	
5/2	1077	1057	560	491	46.5	102	0.62	8,2	11,0	10.35	102	
5/9	1045	1062	592	460	43.3	98	0.65	7,5	14,6	10.50	101	

* Poikilocytosis of red cells.

No previous anemia experiments on this dog.

This experiment (table 44) shows admirably a reaction noted in other experiments. The bleeding reduced the hemoglobin from 123 per cent to 59 per cent and the red cells from 7,500,000 to 3,300,000. During the 6 weeks' observation we note a rise of only 24 per cent hemoglobin but the red count returns to normal. The color index of course drops from 0.93 to 0.58 and poikilocytosis is very much in evidence. Under such conditions one feels a very strong probability of red cell fragmentation.

TABLE 44

Blood regeneration—rice, potatoes and milk. Dog 19–96. Bull mongrel, female, age 8 months

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/17	1520	1238	568	663	53.6	123	0.82	7,5	11,4	13.50	92	
3/17	Diet: Crackermeal and milk											
3/18	Bled 310 cc.											
3/19	Bled 310 cc. No distress											
3/21	527	891	636	246	27.6	59	0.89	3,3	14,2	12.40	72	
3/21	Diet:† 200 grams boiled rice, 200 grams potatoes, 500 cc. milk											
3/28	616	835	551	267	32.0	74	0.93	4,0	14,8	11.55	79	
4/2	662	911	598	308	33.4	73	0.65	5,6	11,4	11.20	81	* Poik.++
4/9	784	959	581	368	38.4	82	0.59	6,9	10,4	10.75	89	* Poik.+
4/16	728	908	550	354	39.0	80	0.56	7,1	6,8	10.30	88	* Poik.+
4/23	818	905	532	368	40.7	90	0.61	7,4	10,2	10.10	90	* Poik.++
4/30	778	938	535	394	42.0	83	0.58	7,2	12,0	9.90	95	* Poik.++

* Poikilocytosis of red cells.

† Animal refused to eat larger quantities of food. Represents about 50 calories per kilo of body weight.

Experimental history, see table 4–b.

This evidence (tables 40 and 44) confirms our belief that the *amount of any diet* may be a considerable factor in blood regeneration. Given a diet of a limited nature but sufficient to permit of slight gain in body weight and we may expect a certain amount of blood regeneration, at times even a return to normal. But given a limited diet in small amounts not sufficient for maintenance of body weight, we may confidently expect a very slow blood regeneration or complete absence of active regeneration. Under such circumstances the body may even be unable to make up its blood cell maintenance factor and the pigment volume curve may actually fall. This is a favorable time for the appearance of a characteristic dietary deficiency disease which is much like scurvy in human beings and is rapidly fatal if not energetically treated with antiscorbutic measures.

TABLE 45

Blood regeneration—casein, sugar, butter and lard. Dog 18-56. Bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
10/29	1275	930	400	530	57	137	0.89	7,7	12,6	8.40	111
10/29	Diet: Bread and milk										
10/30	Bled 233 cc.										
10/31	Bled 233 cc.										
11/2	340	548	400	148	27	62	1.20	2,6	16,0	7.50	73
11/2	Diet: 75 grams casein, 25 grams sugar, 20 grams butter, 20 grams lard										
11/9	428	586	375	211	36	73	1.30	2,8	19,6	7.50	73
11/16	786	827	463	364	44	95	0.99	4,8	6,0	8.10	102
11/23	725	763	412	351	46	95	0.93	5,1	12,0	7.20	106
11/28	908	810	389	421	52	112	0.81	6,9	12,2	7.30	111
12/5	1330	985	384	601	61	135	0.85	7,9	13,2	7.40	133

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
No previous anemia experiments on this dog.

Casein and gliadin. When we consider a bread and milk diet from the standpoint of dietary factors we are obviously dealing with many known and unknown constituents. Two of the familiar ingredients in this bread and milk diet are casein and gliadin, which are concerned particularly in the following group of experiments (tables 45 to 47 inclusive). Casein used in these experiments was obtained from a large dairy products company in this state. It appears as a fine dry granular powder, pale yellow in color, and is of reasonable purity, judging from information given us by the chemist of this company.

The gliadin was extracted from wheat flour in this laboratory by use of dilute alcohol (70 per cent). The weighed amount of gliadin was thoroughly mixed with the sugar, moistened with water and fed to the dog by spoon. Total ingestion was readily accomplished in this way.

Table 45 shows the influence of casein, sugar, lard and butter on blood regeneration. The diet was sufficient to maintain body weight and the blood regeneration was complete in 5 weeks. We must not forget that this dog had not been used for anemia experiments previous to this time and such dogs occasionally show remarkable regenerative capacity on limited diets. The next experiment, however, is conclusive and shows the effect of casein under more carefully controlled and less favorable conditions.

The second casein experiment (table 46) is preceded by a 2 weeks' sugar diet period during which the expected reaction is noted. There is the usual gain in red cell hematocrit, hemoglobin and pigment volume. If the sugar diet had been continued we are reasonably certain that the pigment volume would have remained stationary or even have fallen. Casein added to the diet shows a distinct gain which is held during the subsequent weeks when we see slight fluctuations in pigment volume but relatively little change. There is a slight gain in weight as the calories in the diet are increased by the use of fats. The figures given for the urinary nitrogen show the normal level for the sugar periods and during the sugar and casein intervals indicate the amount of nitrogenous metabolism.

Table 47 is to be compared with a preceding experiment (table 21, paper II). In both experiments a gliadin sugar diet is used over a period of several weeks. This experiment shows less conclusive evidence of the influence of gliadin upon blood regeneration. We may conclude that the gliadin in this experiment (table 47) was without influence on the curve of blood regeneration. We are inclined to the opinion, however, that sugar alone over this period in this experiment would have been associated with a definite loss in pigment volume by the end of the 6-week period. This experiment shows a transient increase in pigment volume which is lost during the last 2 weeks.

TABLE 46

Blood regeneration—casein, sugar, lard and butter. Dog 16-158. Coach mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	AVERAGE DAILY URINARY NITROGEN	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	*grams*	
10/17	1817	1367	588	779	57	133	0.74	8,9	11,0	9.70	141		
10/17	Diet: Bread and milk												
10/19	Bled 342 cc.												
10/21	Bled 342 cc.												
10/22	576	823	576	247	30	70	0.95	3,7	11,2	9.50	87		
10/22	Diet: 75 grams cane sugar, 25 grams glucose, 300 cc. water												
10/29	707	895	573	312	36	79	0.79	5,0	10,6	8.60	104	1.81	
11/5	766	958	575	383	40	80	0.67	6,0	6,0	7.90	121	1.61	
11/5	Diet: 100 grams sugar, 50 grams casein, 300 cc. water												
11/12	856	961	567	394	41	89	0.73	6,1	3,8	7.80	123	4.70	
11/19	1038	1081	616	465	43	96	0.67	7,2	5,2	7.70	140	6.60	Vomiting
11/21	Diet: 100 grams sugar, 100 grams casein, 300 cc. water												
11/26	878	944	538	406	43	93	0.69	6,7	5,0	7.80	121	9.36	
12/3	824	970	582	388	40	85	0.75	5,7	6,2	8.00	121	11.60	
12/3	Diet: 100 grams sugar, 100 grams casein, 10 grams lard, 10 grams butter												
12/10	876	974	604	370	38	90	0.57	7,9	9,8	8.30	117		
12/19	876	952	600	352	37	92	0.69	6,7	14,0	8.70	108		
12/20	Diet: 100 grams sugar, 125 grams casein, 10 grams lard, 10 grams butter												
12/26					40	96	0.61	7,8	9,0	8.90			
1/2/18					38	93	0.55	8,4	7,8	9.10			
1/9					35	92	0.62	7,4	8,6	9.30			
1/17					38	85	0.50	8,5	6,8	9.10			
1/23	975	975	614	361	37	100	0.58	8,7	6,2	8.90	109		

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

TABLE 46-B

Experimental history. Dog 16-158

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 9/26/16	Meat			8.1	
Bled 600 cc				7.7	(4 bleedings)
End 11/20/16				9.5	Complete regeneration of Hb. and R.B.C.
Begin 2/12/17	Beef heart	1523	147	9.9	Table 56
Bled 726 cc.		385	85	9.8	
End 4/11/17		1476	152	8.8	
Begin 5/7/17	Sugar, gliadin	1505	146	9.0	Table 47
Bled 660 cc.	Metabolism	458	84	8.4	
End 6/18/17		456	113	6.2	R.B.C. fragmented, shadow forms
Begin 10/17/17	Sugar	1818	141	9.7	Table 46
Bled 684 cc.	Sugar and casein	576	87	9.5	
End 12/3/17	Metabolism	825	121	8.0	
Begin 5/3/18	Sugar, glycocoll	1800	119	11.7	
Bled 698 cc.	Metabolism	528	76	10.9	
End 6/18/18		852	99	8.05	Maximum regeneration 3 weeks, then drop
Begin 8/28/18	Sugar and gelatin	974	82	10.3	
Bled 929 cc.		548	88	9.15	(5 bleedings)
End 9/30/18		508	90	7.45	Maximum regeneration 2 weeks
Begin 2/20/19	Sugar and liver residue	1862	111	12.35	Table 67
Bled 934 cc.		478	70	11.10	(3 bleedings)
End 3/18/19		738	82	9.15	
Begin 8/18/19	Beet tops	1468	93	12.55	
Bled 851 cc.	Spinach	560	77	12.10	(3 bleedings)
End 10/24/19		785	86	10.50	

These experiments are not conclusive but give evidence that moderate amounts of gliadin with sugar alone do not modify profoundly the blood regeneration curve. That gliadin in combination with other factors may have a more favorable influence on red cell production may be granted. Also under the conditions of the experiment it appears that casein has a more favorable influence on blood regeneration than gliadin when given with sugar only.

TABLE 47

Blood regeneration—sugar and gliadin. Dog 16–158. Coach mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/7	1505	1320	581	740	56	114	0.57	10,1	6,6	9.00	146	
5/8	Bled 330 cc.											
5/9	Bled 330 cc.											
5/11	458	705	480	226	32	65	0.64	5,1	10,8	8.40	84	
5/11	Diet: 50 grams cane sugar, 25 grams dextrose, 25 grams gliadin, 300 cc. water											
5/14	434	804	547	257	32	54	0.68	4,0	9,0	8.20	98	
5/21	435	791	538	253	32	55	0.59	4,7	8,6	7.80	101	*
5/28	596	961	519	346	36	62	0.56	5,5	9,8	7.40	129	
6/4	604	863	552	311	36	70	0.61	5,7	8,4	6.90	122	
6/11	559	766	528	237	31	73	0.61	6,0	9,2	6.60	111	*
6/18	456	702	477	225	32	65	0.53	6,1	11,6	6.20	113	*

* Fragmentation of red blood cells.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Experimental history, see table 46–b.

SUMMARY

A diet of dried white bread and skim milk may cause a slow, steady gain in blood pigment volume from week to week. A liberal diet of this type sufficient to maintain or increase body weight will often suffice for complete blood regeneration. A restricted diet of bread and milk barely sufficient for body maintenance will rarely permit of complete blood regeneration following simple secondary anemia.

Repeat experiments done after short intervals of rest to permit complete return to normal condition will show identical reactions on the part of the hemoglobin, red cells and pigment volume. The animal shows no increased ability to produce hemoglobin and red cells after repeated experiments nor is there any evidence for a failure of red cell production under these conditions.

Bile fistula dogs presenting complete exclusion of bile pigments from the intestine show a reaction which is practically identical with that of normal dogs.

Crackermeal (a mixture of wheat flour, barley flour and rice flour) with milk or lard and butter, gives a blood pigment reaction following anemia which is similar to the familiar bread and milk reaction.

A dietary deficiency disease may develop in these dogs kept on limited diets for many weeks. This condition clinically resembles scurvy in human beings and may be prevented or cured by antiscorbutic measures. This question will be reviewed in a subsequent publication.

Splenectomy may not modify the expected reaction of red blood cells following anemia. In certain splenectomy experiments there develops a peculiar condition associated with spontaneous destruction or disintegration of circulating red cells. This may appear following a limited diet of several weeks and runs a very rapid course resulting in death within a few days.

Rice, potatoes and skim milk make up a diet which may be classed with bread and milk as regards its influence upon red blood cell regeneration following the unit hemorrhages. If anything, this diet is slightly more efficient than bread and milk in promoting blood regeneration.

Casein and gliadin by themselves are not efficient factors in promoting red cell regeneration but casein appears to be the more efficient in the amounts used and under the conditions of these experiments.

Any one of these diet mixtures in proper amounts may be used to maintain the pigment volume at a constant level following the initial 2 weeks' blood reaction. Under such conditions any added food factor may be measured with some accuracy as to its power of aiding in blood regeneration.

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BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA

IV. Influence of Meat, Liver and Various Extractives, Alone or Combined with Standard Diets

G. H. WHIPPLE, F. S. ROBSCHEIT and C. W. HOOPER

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

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Cooked meat and liver stand in striking contrast to the milk, bread, potato mixtures outlined in the preceding paper of this series. Cooked liver, lean beef or beef heart alone or in combination are very efficient in bringing about a rapid blood regeneration following the standard type of secondary anemia. These substances are very similar but for the present we may say their efficiency is in the order given; that is, cooked liver is most effective in anemia provided a sufficient amount (caloric value) is eaten, and cooked beef heart is least effective; but the differences are not great and this order may be changed with the accumulation of more data.

These three substances are efficient in stimulating blood regeneration whether given alone or in combination, or together with carbohydrate or mixed diets. They all stand the severe test of promoting definite blood regeneration when administered after long limited diet periods unfavorable to blood regeneration.

Meat extract (commercial) has no value in the blood regeneration complex. But a watery liver extract seems to exert a distinct influence on the blood regeneration. Liver residue (after the watery and alcoholic extraction) alone exerts a definite influence on blood regeneration. We do not wish to go into a discussion of this question of tissue extracts until we present much more experimental data dealing with this and other material of similar nature.

EXPERIMENTAL OBSERVATIONS

In general the experimental techniqe has been detailed in the first paper of this series. All meat and liver were cooked thoroughly in boiling water before feeding, with the exception of "meat scraps."

The meat scraps were obtained from the University Hospital and included meats of various kinds cooked in different ways. A certain amount of fat was of necessity included in this meat diet. Unless otherwise noted these diets were completely ingested. With an occasional exception noted in the tables the dogs were in uniformly excellent condition.

TABLE 48

Blood regeneration—cooked meat scraps. Dog 17-88. Bull mongrel, female, adult

DATE, 1920	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
2/12	1322	1268	670	591	46.6	104	0.71	7,3	7,4	10.90	115
2/12	Diet: Bread and milk										
2/13	Bled 317 cc.										
2/14	Bled 317 cc.										
2/16	550	1000	714	281	28.1	55	0.75	3,7	6,2	10.35	97
2/16	Diet: 500 grams cooked meat scraps										
2/24	948	1168	713	435	37.2	80	0.69	5,8	10,8	11.05	106
3/1	1046	1236	742	485	39.2	85	0.66	6,4	5.8	11.50	107
3/8	1437	1332	682	636	47.8	108	0.68	7,9	9,8	12.10	110
3/15	1332	1378	734	626	45.4	97	0.62	7,8	7,4	12.85	107
3/22	1475	1420	704	704	49.5	104	0.62	8,4	8,2	13.20	108

Experimental history, see table 20-b.

The first two tables show the characteristic reaction to a diet of meat scraps. There is a prompt and rapid regeneration of hemoglobin and red cells which brings the hematocrit and pigment volume back to practically normal in 3 weeks. This level is sustained for the subsequent 2 weeks. The first experiment (table 48) shows a rather low initial level (104 per cent hemoglobin) but a very prompt reaction following the anemia. The hemoglobin, red cell hematocrit and pigment volume return to a level slightly above the original normal level. There was a marked gain in weight.

The second experiment (table 49) required a third bleeding to reduce the red cells to the usual anemia level. The regeneration of the red cells is practically complete in 3 weeks, although the previous high level of hemoglobin and red cell hematocrit is not reached. This level is uniform during the next 2 weeks. In both experiments the plasma volume is relatively constant during the entire period of observation.

TABLE 49

Blood regeneration—cooked meat scraps. Dog 18–114. Bull mongrel, female, adult

DATE, 1920	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.
2/12	1995	1705	766	931	54.6	117	0.70	8,4	10,8	15.50	110
2/12	Diet: Bread and milk										
2/13	Bled 425 cc.										
2/14	Bled 425 cc.										
2/16	822	1232	818	403	32.7	67	0.98	3,6	7,8	14.30	86
2/16	Bled 275 cc.										
2/18	Diet: 600 grams cooked meat scraps										
2/24	1135	1480	950	504	34.0	77	0.80	4,8	10,2	14.25	104
3/1	1434	1525	857	640	42.0	94	0.73	6,4	9,0	14.35	106
3/8	1580	1540	798	726	47.2	103	0.68	7,6	8,2	15.15	102
3/15	1678	1678	874	796	47.4	100	0.63	7,9	7,8	15.50	108
3/22	1787	1728	838	864	50.0	103	0.63	8,2	9,4	15.50	111

Experimental history, see table 12–b.

Table 50 presents a reaction very much like that noted in the two preceding experiments. Fresh lean beef in adequate amounts was fed. It was cooked in boiling water. This meat contained very little fat and was purchased under the trade name of "chuck." The dog shows a very high level before bleeding and three bleedings did not reduce the level to the usual anemia level. This dog had not been used previously in anemia experiments. The blood regeneration was rapid in the first

2 weeks, but there was little gain in the third week except in the red count, which shows a remarkable jump. This gives a corresponding fall in the color index. A change to a mixed diet shows but little change until the third week of mixed diet when the original high level was attained.

TABLE 50

Blood regeneration—lean beef. Dog 18-24. Bull mongrel, male, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
11/14	1560	1300	480	814	62.6	120	0.87	8,4	12,0	13.20	99
11/12	Diet: Crackermeal and milk										
11/15	Bled 325 cc. Slight distress										
11/16	Bled 195 cc.										
11/18	582	915	612	298	32.6	64				12.70	72
11/18	Bled 150 cc.										
11/20	466	806	539	264	32.7	58	0.81	3,6	20,2	11.85	68
11/20	Diet: 567 grams cooked lean beef										
11/27	935	995	590	399	40.1	94	0.80	5,9	15,8	12.70	78
12/4	1093	1062	585	473	44.5	103	0.61	8,4	14,9	13.15	81
12/11	1090	1033	582	466	45.1	106	0.48	11,9	15,0	13.40	77
12/13	Diet: Mixed diet										
12/20	1055	1115	594	511	45.8	95	0.47	10,1	25,8	13.50	83
12/27	1010	1060	544	516	48.6	95				13.20	80
1/10/19	1425	1230	550	674	54.8	116	0.58	10,1	7,8	13.20	93

No previous anemia experiments on this dog.

Table 51 illustrates a reaction which is in no sense typical, but complicated by abnormal factors. It is submitted with reservations. This dog has been used in a variety of blood regeneration experiments (table 6-b), therefore the type of reaction is well established. But within a few weeks following the present experiment the dog died with bilateral

TABLE 51

Blood regeneration—lean beef and gelatin—lean beef and brain. Dog 17–28. Bull mongrel, female, young adult

DATE, 1918-1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
12/2	2118	1650	740	903	54.7	128	0.58	11,0	9,8	15.70	105	
12/2	Diet: Crackermeal and milk											
12/3	Bled 413 cc.											
12/4	Bled 413 cc.											
12/6	1000	1256	850	394	31.3	79				15.30	82	
12/7	Bled 314 cc.											
12/9	708	1240	926	300	24.2	57	0.79	3,6	15,6	15.05	82	
12/9	Diet: 681 grams cooked lean beef, 20 grams cooked gelatin—100 calories per kilo											
12/16	916	1063	636	388	36.5	86	0.84	5,1	26,2	13.35	80	See footnote
12/17	Diet: 100 grams cooked brain, 580 grams lean beef—100 calories per kilo											
12/23	954	1047	607	412	39.4	91	0.53	8,5	19,0	12.90	81	See footnote
12/23	Diet: Mixed diet											
12/30	883	1100	722	366	33.3	80				13.35	82	
1/8/19	1162	1263	720	531	42.0	92	0.69	6,7	10,2	13.50	94	* Poik. ++
1/15	1050	1290	750	530	41.0	82	0.64	6,4	10,6	14.05	92	*
1/22	1380	1450	792	635	43.8	95	0.64	7,4	17,4	14.65	99	

* Poikilocytosis of red blood cells.

December 16, 1918: Food not touched. Seems sick. Drank considerable water this a.m. and immediately afterward vomited it. Temperature, 38.4°C. Abdomen seems distended. Gave 150 grams meat and 50 grams crackermeal.

December 23, 1918: Left 300 grams food. Very thirsty, vomits water within 5 minutes after drinking. Temperature, 38.8°C. Inactive, slight dragging of right hind leg. Put in metabolism cage; 400 cc. water; mixed diet.

December 26, 1918: Animal recovered. Is still thirsty.

Subsequent death. Stone in kidney.

Experimental history, see table 6–b; see autopsy, table 14.

renal calculi. It is therefore certain that this dog was suffering from renal disease during this experiment (table 51). We note in this experiment that the dog lost weight rapidly on the diet of beef and gelatin which was not eaten with relish. Finally the diet was changed as the beef and gelatin mixture was refused. The beef and brain mixture was also eaten poorly, and more weight was lost. We are fortunate in being able to refer to a fasting experiment on this same dog (table 14) which shows practically an identical gain during two weeks as recorded

TABLE 52

Blood regeneration—beef heart and liver. Dog 18-116. Bull mongrel, female, young adult

DATE, 1918-1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.
12/2/18	2120	1720	805	907	52.7	123	0.56	11,4	14,1	14.90	115
12/2	Diet: Crackermeal and milk										
12/3	Bled 430 cc.										
12/4	Bled 430 cc.										
12/6	939	1183	803	369	31.2	79	0.57	6,9	21,2	14.55	81
12/7	Bled 300 cc.										
12/9	776	1260	930	323	25.6	62	0.84	3,7	17,5	13.95	90
12/9	Diet: 256 grams cooked beef heart,* 610 grams cooked beef liver*—100 calories per kilo										
12/16	1082	1330	844	476	35.8	81	0.88	4,6	18,2	14.55	91
12/23	1890	1685	860	818	48.5	112	0.57	9,9	11,5	15.25	110
12/30	2270	1648	747	902	54.7	138				15.50	106
12/30	Diet: Mixed diet										
1/8/19	2040	1715	785	912	53.2	119	0.72	8,3	7,0	15.50	110
1/15	1760	1610	762	842	52.2	109	0.68	8,0	12,0	16.20	99
1/22	2120	1740	773	948	54.4	122	0.71	8,6	10,6	15.65	111

* Meat cooked, fat and connective tissue removed, and ground.

Experimental history, see table 13-b.

in table 51. The distaste for the food mixture which was eaten only in part we believe is largely responsible for this lack of blood regeneration. We see that the renal disease does not modify the expected blood reaction during a fasting period (table 14) and we have no right to assume that it might seriously modify the reaction in table 51.

TABLE 53

Blood regeneration—beef heart. Dog 19–6. Bull mongrel, male, young adult

DATE, 1918–1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
11/14	2082	1543	590	946	61.3	135	0.77	8,8	11,0	14.00	110
11/14	Diet: Crackermeal and milk										
11/15	Bled 386 cc.										
11/16	Bled 386 cc. No distress										
11/18	800	1020	650	365	35.8	78				13.00	79
11/18	Bled 200 cc.										
11/20	697	1026	710	317	30.8	68	0.74	4,6	16,4	12.80	80
11/20	Diet: 431 grams cooked beef heart*—100 calories per kilo										
11/27	1325	1250	670	574	45.9	106	0.79	6,7	6,0	12.55	100
12/4	1480	1170	568	601	51.1	126	0.64	9,8	6,7	12.75	92
12/11	1642	1325	637	681	51.4	124	0.62	10,5	10,2	12.25	108
12/13	Diet: Mixed diet										
12/20	1376	1188	588	588	49.5	116	0.58	10,0	11,4	12.50	95
12/27	1287	1226	642	579	47.2	105				13.15	93
1/10/19	1940	1462	607	840	57.5	133	0.81	8,2	7,0	13.20	110

* Beef heart cooked, fat and connective tissue removed, and ground.
No previous anemia experiments on this dog.

Table 52 illustrates an optimum reaction on a diet of beef heart and beef liver. Both these substances favor a rapid blood regeneration especially when a sufficient amount is eaten. This dog ate the mixture with relish and gained over 1 kilo during 3 weeks. There is a truly remarkable gain in red cells, hemoglobin and pigment volume. During *3 weeks* the regeneration is complete and the anemia level (3 bleedings) if anything was below the average level. Many other anemia experiments have been completed on this dog (table 13-b) and the type reaction is therefore established. It may be stated even that the regeneration was *almost complete within 2 weeks.*

TABLE 54

Blood regeneration—beef heart. Dog 19–84. Bull mongrel pup, female

DATE, 1918–1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
12/2	992	780	352	424	54.4	127	0.53	12,0	10,6	6.95	112	R. B. C. small
12/2	Diet: Crackermeal and milk											
12/3	Bled 195 cc. No distress											
12/4	Bled 125 cc. No distress											
12/6	448	491	305	183	37.3	91	0.68	6,7	24,0	6.10	80	
12/7	Bled 123 cc.											
12/9	428	542	360	177	32.6	79	1.20	3,3	17,1	5.85	93	
12/9	Diet: 228 grams beef heart*—100 calories per kilo											
12/16	638	608	332	274	45.0	105	0.86	6,1	10,1	5.80	105	
12/23	796	676	328	345	51.0	118	0.59	10,8	15,2	5.90	115	R. B. C. small
12/30	968	711	323	387	54.5	136				5.90	120	
12/30	Diet: Mixed diet											
1/8/19	855	750	366	380	50.5	114	0.71	8,0	23,8	6.95	107	
1/15	712	818	405	409	50.0	87	0.61	7,1	10,8	7.80	105	
1/22	1020	937	476	456	48.7	109	0.55	10,5	14,2	7.50	125	
1/30	987	888	429	455	51.2	111	0.75	7,4	11,2	7.50	118	

* Beef heart cooked, fat and connective tissue removed, ground.

No previous anemia experiments on this dog.

With the change to mixed diet we note a reaction which is not uncommon when a sudden change is made from a fixed diet to another very different diet. Under such conditions even when the second diet is most favorable we may record a slight fall in red cell hematocrit, hemoglobin and pigment volume. This experiment, too, shows a fall in the red count. We have no good explanation to offer, but this fact is to be considered in the proper interpretation of various tables.

TABLE 55

Blood regeneration—beef heart and lean meat following 3 weeks of sugar—metabolism. Dog 16–157. Bull mongrel, male, age 10 months

DATE, 1916–1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.	
12/14/16						116		7,9	6,4	8.10		
12/14	Diet: Sugar (3 weeks)											
1/5/17	546	976	625	351	36	56	0.55	5,1	7,2	6.10	160	
1/5	Diet: 400 grams beef heart, 100 grams sugar											
1/11					43	76	0.58	6,6	11,8	6.60		
1/13	Diet: Lean meat											
1/17	408	908	563	345	38	45	0.44	5,1	6,4	7.60	119	*
1/26	738	838	478	360	43	88	0.59	7,5	8,2	8.00	105	*
2/2	838	998	529	469	47	84	0.57	7,3	7,0	8.20	122	
2/16	1130	1202	553	649	54	94	0.53	8,8	9,8	9.20	131	
2/23	1052	1052	526	526	50	100	0.58	8,6	13,4	9.20	115	
3/2	1208	1220	549	672	55	99	0.56	8,9	9,8	9.20	132	

* Anisocytosis and poikilocytosis of red blood cells.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Cooked beef heart is a food commonly used in experimental laboratories. It is usually assumed that heart muscle as a food is very like skeletal muscle, although there may be certain differences as pointed out by Mendel and Osborne (1). We have found that it compares favorably with skeletal muscle as far as concerns the regeneration of red cells and hemoglobin. Whether beef heart is actually identical with lean beef in its effect on blood regeneration cannot be stated positively as we cannot at this time submit a sufficiently complete series of controlled experiments.

Tables 53 and 54 are identical in all essential factors. Both dogs were used for the first time and we must consider the unusual" reserve" which at times may be demonstrated by such dogs. The cooked beef heart was eagerly eaten, and the weight was practically stationary, although the first dog lost a little during the third week. Blood regeneration was practically complete in 3 weeks as regards red cells, hemoglobin and pigment volume. Both dogs showed a distinct drop when changed to a mixed diet,—a change recorded in red cell hematocrit, hemoglobin and red count. There was no change in plasma volume to explain this fluctuation and for the present we must be content with recording this fact without advancing any convincing explanation (refer to exper. 52). A similar fall is noted in table 66 even when the change is from a poor diet (bread and milk) to a more favorable diet (beef heart).

TABLE 55-B

Experimental history. Dog 16-157

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 9/26/16	Fasting			7.90	
Bled 600 cc.				6.80	(4 bleedings)
End 10/11/16				5.40	Slight regeneration of Hb. and R. B. C.
Begin 12/14/16	Sugar, metabolism			8.10	
Bled 600 cc.		1208	132	7.30	(4 bleedings)
End 3/2/17	Sugar, beef heart			9.20	
	Beef heart				
Begin 5/7/17	Gelatin, sugar	1570	130	10.00	
Bled 648 cc.	Metabolism	429	74	9.20	
End 6/11/17		792	126	6.30	
6/14	Killed—bled from carotid				

Table 55 illustrates the reaction on beef heart diet following a 3-week period of sugar feeding. After the meat diet is established we note a slow but steady gain back to normal in 4 to 5 weeks. We wish to emphasize the fact that a period of fasting or sugar feeding makes subsequent blood regeneration much more difficult and this is a severe test for any food substance. Only meat (including beef heart) and liver show up to advantage as compared with the mixed diet under such conditions. Apparently the fasting or sugar feeding or other limited diet causes a draining of the body's reserve and subsequent blood regeneration suffers because of this depletion of reserve or impairment of function.

TABLE 56

Blood regeneration—beef heart. Dog 16–158. Coach mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.
2/12	1525	1451	566	886	61	105	0.63	8,3	7,0	9.90	147
2/12	Diet: Bread and milk										
2/13	Bled 363 cc.										
2/14	Bled 363 cc.										
2/15	385	837	560	276	33	46	0.70	3,3	7,4	9.80	85
2/15	Diet: Beef heart										
2/21	500	980	647	333	34	51	0.77	3,3	10,8	9.70	101
2/28	602	885	523	363	41	68	0.68	5,0	19,8	9.80	90
3/7	840	1077	560	517	48	78	0.56	6,9	19,8	9.80	109
3/14	904	1062	531	531	50	85	0.57	7,5	14,0	9.30	114
3/21	1235	1272	585	687	54	93	0.60	7,8	10,0	9.50	134
3/28	1146	1180	543	638	54	97	0.58	8,3	8,0	9.50	124
4/5	1280	1243	572	671	54	103	0.56	9,2	8,6	9.30	134
4/11	1478	1342	604	738	55	110	0.58	9,4	18,6	8.80	152

Experimental history, see table 46–b.

Tables 56 and 57 are incomplete in that the amount of beef heart is not known but the weights give assurance that a liberal amount of food was consumed. There is only a trifling loss of weight during the last week of the experiment when the blood picture had returned to normal. It is obvious that the blood regeneration in these two experiments is much slower than that recorded in experiments 53 and 54. Four to 6 weeks elapse before the hemoglobin and hematocrit return to normal and 5 to 7 before the pigment volume and red cell count return to the original level.

TABLE 57

Blood regeneration—beef heart. Dog 17–192. Bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
4/24	1157	1032	537	496	48	112	0.78	7,2	10,6	9.80	105
4/24	Diet: Bread and milk										
4/25	Bled 258 cc.										
4/26	Bled 258 cc.										
4/27	444	822	633	189	23	54	0.84	3,2	12,8	9.50	87
4/27	Diet: Beef heart										
5/2	662	883	609	274	31	75	0.79	4,7	11,6	9.90	89
5/9	804	992	585	407	41	81	0.67	6,0	8,0	9.50	104
5/16	1030	1050	567	483	46	98	0.80	6,1	12,0	9.50	110
5/23	1255	1172	633	539	46	107	0.73	7,3	9,0	9.50	123
5/30	1308	1147	585	562	49	114	0.81	7,0	8,0	9.50	121
6/6	1360	1214	607	607	50	112	0.64	8,7	6,8	9.10	133

No previous anemia experiments on this dog.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Tables 58 and 59 both deal with bile fistula dogs. The experiments were performed at the same time under identical conditions and the reaction to the beef heart diet is strikingly uniform. It is known from the autopsy notes that the bile was completely excluded from the intestine. A meat diet is not well tolerated by these bile fistula dogs over long periods and a loss of weight is usually noted under such conditions. As a result of this long period of meat feeding we note subsequent intoxication which resulted fatally in spite of a mixed diet régime.

The beef heart was eaten with relish but the amounts given are not recorded. There is a distinct gain in hemoglobin, red cells and pigment volume during each of the first 3 weeks. The level at the end of the third week is almost normal in both dogs and if the experiments had been terminated at this point we should of necessity conclude that the reaction was similar to that so often observed in the normal dog.

TABLE 58

Blood regeneration—beef heart—bile fistula. Dog 17-35. Bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/28	1330	1244	560	684	55	107	0.74	7,2	20,0	9.10	136	
5/29	Bled 311 cc.											
5/30	Bled 311 cc.											
5/31						48	0.77	3,1	45,6	9.10		
5/31	Diet: Beef heart											
6/8	540	900	567	333	37	60	0.79	3,8	14,6	8.20	109	
6/15	868	923	480	443	48	94	0.78	6,0	7,0	8.40	109	
6/22	952	933	476	457	49	102	0.72	7,1	8,4	8.10	115	
6/29	800	1000	590	410	41	80	0.74	5,4	6,8	8.40	119	No increase in bile pigment
7/6	649	729	459	277	38	89	0.78	5,7	7,0	8.00	91	
7/9	Diet: Mixed diet											
7/13	694	846	533	313	37	82	0.77	5,3	20,0	8.80	96	
7/18	797	848	500	348	41	94	0.90	5,2	19,8	9.00	94	
8/9	Death from bile fistula intoxication											

Bile pigment daily output per 6 hours before this experiment (28 day average) = 14.0 mgm.

Bile pigment daily output per 6 hours during this experiment (30 day average) = 13.4 mgm.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

But the fourth week in both dogs shows a decided drop in pigment volume, hemoglobin and red cell hematocrit.

We fortunately have the daily *bile pigment output figures* at hand and can say that there is no increase in bile pigment elimination during this week of falling hemoglobin. So we may not explain this decrease in the curve as due to some agent destructive to the red cells. It has been established (2) that a sudden destruction of red cells in the blood stream will result in an increased output of bile pigment although the reaction is not in any degree a quantitative reaction as some observers have claimed (3). We have no convincing explanation for these observed facts but suggest that the poor quality of the red cell may be a factor. Limited diets as well as splenectomy under certain experimental conditions seem to be associated with red cells which are prone to disintegrate more readily than normal.

TABLE 58–B

Experimental history. Dog 17–35 (bile fistula)

EXPERIMENT	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram		
				kgm.	
Begin 12/18/16	Lean meat			8.80	100 Hb.
Bled 600 cc.				8.90	(4 bleedings)
End 1/17/17		892	99	8.90	101 Hb.
3/8/17	Bile fistula operation				
Begin 5/28/17	Beef heart	1330	136	9.10	107 Hb.
Bled 622 cc.				9.10	
End 7/6/17		649	91	8.00	Maximum regeneration 3 weeks

The average daily bile pigment output is given in each table for a 30-day period before the experiment and during the anemia regeneration period. One dog shows identical figures for the mixed diet control and beef heart period. The other dog shows a much higher output on the mixed diet than on the beef heart diet. We believe this is to be explained by the mixed diet which is made up of meat scraps, bones, bread, potatoes, table scraps, etc., and is given in moderate excess. This allows a certain choice on the part of the dog and if the animal prefers the carbohydrate fractions we may observe the familiar reaction to carbohydrate feeding in the bile fistula dog, namely, an increase in bile pigments.

TABLE 59

Blood regeneration—beef heart—bile fistula. Dog 17–155. Bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.	
5/28	1560	1416	708	708	50	110	0.67	8,2	11,2	11.50	123	
5/28	Diet: Mixed diet											
5/29	Bled 354 cc.											
5/30	Bled 354 cc.											
5/31	495	935	645	290	31	53	0.63	4,2	9,2	10.80	86	
5/31	Diet: Boiled beef heart											
6/8	1030	1213	752	461	38	85	0.70	6,1	9,0	10.90	111	
6/15	1165	1265	645	620	49	92	0.66	7,0	10.4	10.90	116	
6/22	1385	1281	615	666	52	108	0.68	7,9	9,8	10.00	128	
6/29	946	1186	700	484	41	80	0.67	6,0	14,6	9.80	121	No bile pigment increase
7/6	1100	1038	571	468	45	106	0.73	7,3	15,0	10.00	103	
7/13	1138	1138	683	455	40	100	0.86	5,8	14,4	9.90	115	
7/18	1120	1288	824	464	36	87	0.84	5,2	9,8	10.30	125	No bile pigment increase
7/19	Diet: Mixed diet											
8/9	Death from bile fistula intoxication											

Bile pigment daily output per 6 hours before this experiment (30 day average) = 21.8 mgm.

Bile pigment daily output per 6 hours during this experiment (30 day average) = 15.2 mgm.

No previous anemia experiments on this dog.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

We may recall that the fasting bile fistula dog will react as promptly to anemia as the normal dog (table 23) and the bile fistula dog on a bread and milk diet also presents a normal blood regeneration curve (table 32). But the meat diet reaction in the bile fistula dog is not like the reaction of the normal animal. The unfavorable clinical reaction of the bile fistula dog to the meat diet is often conspicuous (diarrhea and loss of weight and activity) and this may explain the observations recorded in tables 58 and 59.

TABLE 60

Blood regeneration—liver. Dog 19–83. Bull mongrel pup male

DATE, 1918–1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.	
12/2/18	1090	1001	540	466	46.1	108	0.59	9,1	8,8	9.30	109	R. B. C. small
12/2	Diet: Bread and milk											
12/3	Bled 250 cc.											
12/4	Bled 250 cc.											
12/6	489	790	586	202	25.5	62	0.63	4,9	13,8	8.80	90	* Slight
12/7	Bled 200 cc.											
12/9	630	1032	770	258	25.0	61	1.20		13,7	8.65	119	
12/9	Diet: 550 grams cooked beef liver†—100 calories per kilo											
12/16	873	910	536	370	40.6	96	0.94	5,1	11,4	8.85	103	
12/23	962	962	550	407	42.3	100	0.56	8,9	19,2	9.35	103	
12/30	1304	1100	566	530	48.1	118				9.90	111	
12/30	Diet: Mixed diet											
1/8/19	1300	1235	642	580	47.0	105	0.80	6,6	14,2	10.75	115	
1/15	1095	1200	670	519	43.2	91	0.75	6,1	12,8	11.40	105	
1/22	1355	1300	685	600	46.2	104	0.75	6,9	8,0	11.30	115	

*Poikilocytosis of red blood cells.

† Beef liver cooked, fat and connective tissue removed, ground.

No previous anemia experiments on this dog.

Tables 60 and 61 show the remarkable influence which cooked liver exerts upon blood regeneration. As the sole article of food cooked liver may not be well tolerated, but in these two experiments the dogs ate all of the liver and gained weight. The remarkable gain in hemoglobin, red cells and pigment volume is at once obvious at a glance. One experiment (table 61) shows practically complete regeneration in 2 weeks from the usual anemia level and both dogs are more than back to normal in 3 weeks. We are able to refer to a number of other experiments on this dog (table 61, dog 18-114, exper. history table 12-b) to give a good line on the type normal blood regeneration.

TABLE 61

Blood regeneration—liver. Dog 18–114. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
11/14	1640	1411	643	763	54.0	116	0.58	11,3	14,8	13.50	105
11/14	Diet: Crackermeal and milk										
11/15	Bled 353 cc.										
11/16	Bled 353 cc. No distress										
11/18	852	1077	695	372	34.5	79				12.85	84
11/18	Bled 270 cc. No distress										
11/20	592	1015	724	280	27.6	58	0.82	3,5	14,2	12.50	81
11/20	Diet: 750 grams cooked beef liver*										
11/27	1305	1290	726	540	41.8	101	0.74	6,8	11,8	13.25	97
12/4	1730	1480	740	720	48.5	117	0.76	7,7	20,0	13.35	111
12/11	1902	1516	728	766	50.5	125	0.82	7,6	13,4	13.65	111
12/13	Diet: Mixed diet										
12/20	1479	1333	713	613	46.0	111	0.65	8,6	14,6	13.90	96
12/27	1295	1276	670	594	46.5	101				13.75	93
1/10/19	1625	1390	668	716	51.5	117	0.68	8,6	12,8	14.20	98

* Beef liver cooked, fat and connective tissue removed, ground.

Experimental history, see table 12-b.

Table 62 shows another experiment with cooked beef liver but the amount of liver fed is much less and the rest of the food caloric value is made up by crackermeal and milk. We see this small amount of liver causing a prompt rise of the hemoglobin and red cells to normal in 3 weeks, whereas the crackermeal and milk alone would require at least 5 to 6 weeks for complete regeneration.

TABLE 62

Blood regeneration—liver and crackermeal. Dog 19–15. Brindle bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/9	1702	1120	462	650	58.0	152	0.95	7,9	6,2	10.85	103	
8/9	Diet: Crackermeal and milk											
8/12	Bled 280 cc. No distress											
8/13	Bled 280 cc. No distress											
8/15	663	762	501	253	33.2	87				10.40	73	
8/15	Bled 190 cc. No distress											
8/17	468	669	493	173	25.8	70	0.92	3,8	16,8	10.35	65	* Poik. ++
8/17	Diet: 70 grams cooked beef liver,† 200 grams crackermeal, 500 cc. milk											
8/23	664	874	517	267	30.5	76	0.88	4,3	15,0	10.85	81	
8/30	1290	949	466	478	50.3	136	0.90	7,5	15,8	11.30	84	
9/6	1600	1096	504	588	53.6	146	0.92	7,9	8,2	11.20	98	
9/13	1700	1089	495	594	54.5	156	0.96	8,1	10,8	11.50	95	

* Poikilocytosis of red blood cells.

† Beef liver cooked, fat and connective tissue removed, ground.

No previous anemia experiments on this dog. See table 64 for subsequent experiment.

Table 63 is not very satisfactory but is included because several interesting points may be made. This dog at the beginning of the experiment was very young, approximately 4 months, but the date of birth was not positively known. The pup had a hemoglobin of 73 per cent, which is not unusual in young dogs of this age. The amounts bled were less than normal because of this fact, but the total reserve was evidently considerable, as indicated by the high figures after the bleeding. The young dog was growing rapidly during the whole period of the experiment, a gain of 3 kilos body weight in 6 weeks. The blood volume shows a considerable increase during this period, almost 100 per cent gain. The gain in hemoglobin and hematocrit is steady and comes close to the average normal at the end of the experiment, in marked contrast to the level at the beginning of the experiment.

TABLE 63

Blood regeneration—liver and bread. Dog 18–114. Bull mongrel pup, female

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
4/24	553	758	455	291	38.4	73	0.53	6,9	23,6	7.85	94
4/24	Diet: Bread and milk										
4/26	Bled 189 cc.										
4/27	Bled 90 cc. No distress										
4/29	435	805	528	255	31.7	54	0.58	4,7	14,6	7.65	105
4/30	Diet: 200 grams bread, 500 cc. milk, 50 grams cooked beef liver,* ground up with bread										
5/8	599	740	432	293	39.6	81	0.56	7,2	8,4	8.00	93
5/15					43.6	88	0.52	8,5	20,6	8.80	
5/22	825	938	514	411	43.8	87	0.54	8,0	16,0	9.50	98
5/29	791	807	463	330	40.9	98	0.69	7,1	22,8	9.60	84
5/29	Diet: Mixed diet										
6/5		902	497	391	43.4					10.45	86
6/10	1059	1009	556	443	43.9	105	0.67	7,8	11,8	10.70	94

* Beef liver cooked, fat and connective tissue removed, and ground up with bread.

Experimental history, see table 12–b.

No previous anemia experiments on this dog.

Meat extract (tables 64 and 65) evidently does not add anything to a given diet which in itself is especially favorable to blood regeneration. We have no evidence to show that meat extract is favorable or unfavorable to blood reconstruction. Table 64 shows the rapid blood regeneration which we expect on a cooked liver diet and the return to normal requires but 3 weeks. We note again in this experiment the fall which often occurs following a sudden change to another diet (mixed diet) which, too, is very favorable for blood regeneration and maintenance. It is clear that there is no fluctuation in plasma volume which would supply an easy explanation for this phenomenon.

TABLE 64

Blood regeneration—liver and beef extract. Dog 19–15. Brindle bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.
11/14	1615	1260	506	746	59.3	128	0.76	8,4	10,8	12.40	102
11/14	Diet: Crackermeal and milk										
11/15	Bled 315 cc. No distress										
11/16	Bled 315 cc. No distress										
11/18	584	808	550	255	31.5	72				11.95	68
11/18	Bled 202 cc.										
11/20	508	796	568	224	28.1	64	0.69	4,6	14,4	11.35	70
11/20	Diet: 450 grams cooked beef liver* and 10 grams Liebig's beef extract										
11/27	808	917	565	347	37.9	88	0.76	5,8	12,8	11.30	81
12/4	1100	1002	555	458	45.0	108	0.66	8,2	15,3	11.50	89
12/11	1300	1084	560	518	47.8	120	0.60	10,3	12,2	11.35	95
12/13	Diet: Mixed diet										
12/20	912	954	550	395	41.6	96	0.56	8,6	16,8	11.95	80
12/27	926	1010	564	427	42.2	92				11.85	85
1/10/19	1122	1060	540	508	48.0	106	0.91	8,0	13,6	12.00	88

* Beef liver cooked, fat and connective tissue removed, ground.

Refer to table 62 for previous experiment.

Meat extract (table 65) does not modify the reaction which may be expected following a liberal bread and milk diet. The blood picture is returned almost to normal in 4 weeks after the anemia and there is a gain in weight of 1 kilo. A change to a mixed diet gives a favorable reaction as is usual under these circumstances. We may refer also to a part of table 66, which gives more data on the influence of bread and milk plus meat extract. The meat extract adds nothing to the reaction which is identical with the expected reaction from the bread and milk alone.

TABLE 65

Blood regeneration—meat extract—bread and milk. Dog 18-116. Bull mongrel pup, female

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX*	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.	
4/24	903	962	459	486	50.5	94	0.65	7,2	9,8	9.75	99	* Slight
4/24	Diet: Bread and milk											
4/25	Bled 240 cc. from jugular vein											
4/26	Bled 190 cc. from jugular vein											
4/29	422	862	594	259	30.0	49	0.57	4,3	10.2	9.65	89	* Slight
4/29	Bled 110 cc. from jugular vein											
4/30	Diet: 200 grams bread, 500 cc. milk, 10 grams Liebig's beef extract											
5/8	717	956	591	347	36.3	75	0.48	7,8	10,0	9.60	99	*
5/15	882	1297	760	517	39.9	68	0.46	7,3	8,6	9.50	137	*
5/22	859	1035	594	430	41.5	83	0.51	8,2	22,8	10.70	97	*
5/29	865	961	550	403	41.8	90	0.54	8,3	10,2	10.85	89	*
5/29	Diet: Mixed diet											
6/10	1207	1128	634	483	42.8	107	0.63	8,5	30,0	12.50	90	
6/18						104						

* Poikilocytosis of red blood cells.
Experimental history, see table 13-b.

Tables 66 and 67 are to be considered together. The experiments were done at the same time under identical conditions and the results

TABLE 66

Blood regeneration—watery liver extract and sugar; bread and milk; meat extract, bread and milk; beef heart, bread and milk. Dog 17–157. Coach mongrel, female, young adult

DATE, 1910	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
2/20	1340	1180	567	606	51.4	113	0.79	7,2	8,2	10.35	114	
2/20	Diet: Bread and milk											
2/21	Bled 295 cc. No distress											
2/22	Bled 295 cc. Distress. Injected 50 cc. of 5 per cent sugar solution											
2/24	420	806	602	198	24.6	52	0.96	2,7	8,6	9.50	85	
2/26	Diet: 100 grams sugar, 10 grams watery liver extract,† 250 cc. water											
3/5	720	868	519	345	39.7	83	0.90	4,6	11,0	8.95	97	
3/12	700	813	493	312	38.4	86	0.88	4,9	7,2	8.25	99	
3/18	666	730	420	294	40.2	91	0.80	5,7	8,8	8.05	91	
3/18	Diet: 200 grams bread, 300 cc. milk											
3/26	824	929	560	360	38.7	89	0.85	5,2	10,0	8.40	110	
3/26	Diet: 200 grams bread, 300 cc. milk, 10 grams commercial meat extract											
4/2	756	913	556	398	38.1	83	0.90	4,6	10,8	8.75	104	
4/8	635	829	522	298	36.0	77	0.67	5,7	14,0	8.75	95	*
4/14	714	876	538	324	37.0	82	0.73	5,6	11,2	8.75	100	
4/21	860	984	584	389	39.6	87	0.72	6,0	12,8	8.90	110	
4/21	Diet: 200 grams beef heart (cooked), 200 grams bread, 300 cc. milk											
4/28	772	942	588	345	36.6	82	0.67	6,1	12,4	9.55	99	
5/7	842	961	572	384	40.0	88	0.73	6,0	15,4	10.00	96	
5/12	890	998	590	397	39.8	89	0.72	6,2	15,0	9.90	101	
5/12	Diet: Mixed diet											

* Poikilocytosis of red blood cells.

† Watery liver extract: Beef liver cut up into small cubes, allowed to stand in water over night in ice-chest, boiled in same water, and filtered. Filtrate concentrated to thick paste.

TABLE 66-B

Experimental history. Dog 17–157

EXPERIMENT NUMBER	DIET	BLOOD REGENERATION		WEIGHT	REMARKS
		Pigment volume	Blood per kilogram	kgm.	
Begin 9/3/17 Bled 496 cc. End 10/25/17	Crackermeal, lard and gelatin	1025 380 890	115 83 102	8.40 8.20 8.70	
Begin 3/6/18 Bled 488 cc. End 4/9/18	Crackermeal, lard, butter and Blaud's pills	1084 614 984	99 90 106	9.80 9.10 8.30	Table 71
Begin 5/20/18 Bled 612 cc. End 6/18/18	Sugar, metabolism, desiccated beef heart	1113 313 663	120 75 100	10.20 9.50 7.60	Maximum regeneration 3 weeks. Pigment volume 698 cc.
Begin 8/28/18 Bled 710 cc. End 11/12/18	Hb. and sugar feeding Hb. intravenously and sugar feeding Hb. intravenously and crackermeal + milk Crackermeal, milk and dried yeast	1366 433 886	102 79 114	10.10 9.25 8.15	Table 78 (3 bleedings) Pigment volume 805 at end of Hb. period
Begin 2/20/19 Bled 590 cc. End 5/12/19	Liver extract, sugar Bread, milk, meat extract Beef heart, bread and milk	1340 420 890	114 85 101	10.35 9.50 9.90	Table 66 Pigment volume 666 cc. at end of sugar feeding

are very suggestive. Both dogs have been observed in many other experiments and their anemia reactions are therefore well known. One dog was given the watery extract of beef liver and the other the liver residue. Sugar, 100 grams, was added to each feeding and it is clear

TABLE 67

Blood regeneration—liver residue and sugar; bread and milk; crackermeal and bread and milk; cooked beef liver and bread and milk. Dog 16–158. Coach mongrel, male, young adult

DATE, 1919	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.	
2/20	1862	1375	549	820	59.6	135	0.77	8,8	7,0	12.35	111	
2/20	Diet: Bread and milk											
2/21	Bled 344 cc. No distress											
2/22	Bled 344 cc. Dyspnea; injected 50 cc. of 5 per cent sugar solution											
2/24	823	983	625	353	35.9	84	1.02	4,1	19,6	11.40	86	
2/25	Bled 246 cc.											
2/27	478	778	562	211	27.2	61	0.85	3,6	8,4	11.10	70	
2/27	Diet: 100 grams sugar, 200 cc. water by stomach tube; 100 grams liver residue†											
3/5	692	867	531	331	38.2	80	0.76	5,3	6,4	10.75	81	*
3/12	876	900	517	373	41.5	97	0.84	5,8	5,4	9.95	90	* Poik.+
3/18	738	754	421	329	43.7	98	0.83	5,9	14,4	9.15	82	* Poik.+
3/18	Diet: 200 grams bread, 300 cc. milk											
3/26	725	923	597	316	34.3	79	0.77	5,1	6,8	10.15	91	* Poik.
3/26	Diet: 200 grams bread, 100 grams crackermeal, 300 cc. milk											
4/2	748	944	600	339	35.9	79	0.61	6,5	9,8	10.50	90	* Poik.+
4/8	685	966	616	340	35.2	71	0.61	5,8	10,8	11.10	87	* Poik.
4/14	762	968	608	355	36.7	79	0.59	6,7	7,8	12.75	76	* Poik.
4/21	840	1072	640	406	37.9	78	0.56	7,0	10,6	11.20	96	
4/21	Diet: 200 grams cooked beef liver, 200 grams bread, 300 cc. milk											
4/28	820	1027	629	388	37.8	80	0.73	5,5	12,0	11.95	86	
5/7	1155	1155	595	549	47.5	100	0.75	6,7	9,6	11.45	101	
5/12	1074	1095	583	502	45.8	98	0.66	7,4	13,0	11.80	93	* Poik.+
5/12	Diet: Mixed diet											

* Poikilocytosis of red blood cells.

† Liver residue: Residue left after water and alcoholic extraction; put in meat press and all liquid removed. Just before feeding residue was again washed and brought to boiling point to remove all traces of alcohol. Dog did not always eat full amount of food mixture.

Experimental history, see table 46–b.

that both the liver watery extract and liver residue exert a certain influence upon the blood regeneration which is much more than can be accounted for by the sugar alone. The liver residue has greater influence upon the blood regeneration than does the liver watery extract but the difference is not striking.

Bread and milk feeding for 1 week subsequent to these sugar and liver periods does not cause much reaction. There is a slight loss in hemoglobin and red cell hematocrit in experiment 67 (liver residue).

The next 4 weeks are similar and bread or crackermeal and milk are the essential features of the diet. The level of pigment volume, red cell hematocrit and hemoglobin is constant. There is slight gain in the red cell counts.

We have pointed out the fact that a prolonged diet period unfavorable to hemoglobin regeneration will leave the dog in a condition which may be clinically excellent but from the standpoint of formation of hemoglobin very unfavorable. These two dogs were still anemic although in excellent physical condition and of practically normal weight. Under such circumstances we feel that any diet is given its most severe test and few diets of limited nature can give a favorable reaction as regards blood regeneration. Under ordinary circumstances a diet of beef heart, bread and milk (table 66) is favorable for blood regeneration but under this severe test with unfavorable conditions we note little if any blood regeneration during a period of 3 weeks.

Cooked beef liver with bread and milk even under these same unfavorable conditions is able to effect a prompt blood regeneration—conspicuous in red cell hematocrit, red count and hemoglobin. This is the severest test of any diet factor in its relation to blood regeneration. Also refer to table 22 (same experiment).

DISCUSSION

The reader will observe many individual differences in the reactions of various dogs to a unit type of secondary anemia. Some of these vagaries we are as yet unable to explain but others are now much clearer than in the earlier stages of this investigation. We have noted that on occasions certain dogs *made anemic* for the *first time* presented a most unusually rapid regeneration, even on a very limited diet. This is not the rule, but is sufficiently common to suggest caution in conclusions drawn from such experiments. A repeat experiment will give the true constant reaction. How to explain this fact is not clear to us, but a simple way out is to assume a reserve present in the body under these conditions which permit of unusual blood regeneration even under most unfavorable diet conditions. A knowledge of this fact may keep the investigator from falling into error in deductions drawn from single experiments. With this exception the dogs will show uniform reactions when we repeat anemia experiments under uniform conditions.

It is clear that long limited diet periods following the standard anemia may preserve the dogs in excellent physical condition as concerns weight, general condition and activity. These same dogs, however, may continue to present a definite anemia. Under such conditions many diet mixtures are unable to stimulate any blood regeneration. Any diet factors are put to the severest test when they are administered to dogs under such circumstances and we are inclined to accept this as the severest test for any given diet factor. Cooked liver gives a very favorable reaction and causes blood regeneration even under these severe test conditions.

Following a simple anemia many diet mixtures, if given at once, will cause a distinct gain in hemoglobin and red cells. But if a limited diet period intervenes between the anemia and the exhibition of the test diet we will see a negative reaction. This may be illustrated by bread and milk given in *liberal amounts* sufficient to permit a gain in body weight or at least a maintenance of body weight. If the dog is bled and at once placed on a bread and milk diet there will usually appear a slow steady gain in pigment volume. If the same dog is placed on a limited *carbohydrate diet* for 3 to 4 weeks before being changed to a liberal bread and milk diet we will usually observe subsequently an unchanged level of pigment volume, red cell hematocrit and hemoglobin. Under such unfavorable conditions the bread and milk diet is unable to give a favorable reaction for the blood regeneration. In certain experiments the cooked beef heart is also unable to modify the curve of blood regeneration under these unfavorable conditions. Cooked liver is able to induce blood regeneration even under the most unfavorable conditions. The same is true for the common *mixed diet* of table scraps. It is important to keep these facts in mind when we evaluate the reaction following the administration of a given diet factor under different experimental conditions.

The question at once confronts us: what part of the meat or liver substance is responsible for the favorable blood reaction? First we must investigate the pigment substances present in the meat—for example, the hemoglobin and myohematin. Some experiments with hemoglobin appear in the next paper of this series, and we hope soon to report other experiments dealing with the myohematin pigment.

SUMMARY

Cooked lean beef and beef heart are diet factors of importance as regards blood regeneration subsequent to simple secondary anemia. These food substances alone or in combination with other foods will give a rapid blood regeneration after anemia.

Anemia is produced by bleeding one-fourth of the measured blood volume on each of 2 successive days. This anemia will be completely repaired within 3 to 4 weeks if the dog is given a liberal diet of meat or beef heart.

Cooked liver is as sufficient as meat and may be even more efficient in promoting complete blood regeneration subsequent to a standard anemia. Blood regeneration may be completed in 2 to 4 weeks.

Commercial meat extract is inert and watery liver extract has but little influence upon blood regeneration.

The meat diet reaction in the bile fistula dog is not exactly like the reaction of the normal animal.

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BLOOD REGENERATION FOLLOWING SIMPLE ANEMIA

V. THE INFLUENCE OF BLAUD'S PILLS AND HEMOGLOBIN

C. W. HOOPER, F. S. ROBSCHEIT AND G. H. WHIPPLE

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

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The familiar fact that iron in some form is very frequently given in cases of secondary anemia made it imperative to test this drug under a variety of experimental conditions. The outstanding fact in our experiments is that *iron given as Blaud's pills has no influence in secondary anemia under the conditions of these experiments.* It may be objected that the Blaud's pills were not fresh or were not dissolved in the dog's intestinal tract. We obtained these Blaud's pills from a large wholesale firm in this city and they were soft and easily crushed into a soft, pasty material. The pills were crushed before being given by mouth. Further objections may be made that this drug has no influence on the dog but does have potency when administered to human beings. This of course is not subject to proof, but the claims for the potency of iron in conditions of secondary anemia do not stand on firm ground. We invite attention to the profound influence which is properly attributed to diet factors. Those who claim that iron is a potent drug must exclude the food factors which are known to be concerned before they can prefer too many objections to our negative results.

The feeding of blood has at times been used in the treatment of secondary anemia. We are able to find some experimental evidence to support this treatment, but whole red cells or hemoglobin given by mouth in the form of a dry powder do not appear to influence profoundly the blood regeneration curve. Our experiments show that hemoglobin does have a distinct influence on blood regeneration but not sufficient to warrant its use in uncomplicated secondary anemia in view of the favorable reactions due to meat and other diet factors.

The favorable reaction which seems to accompany administration of hemoglobin by injection (intravenous and intraperitoneal) may be of some value in the treatment of certain forms of anemia. It is possible that the reaction to this type of injection may differ from that associated with a transfusion and in certain diseases this procedure (hemoblobin injection) may stimulate rather than depress the bone marrow. Further experimental work is in progress.

EXPERIMENTAL OBSERVATIONS

The same technical procedures are used in these experiments as have been described in the first paper of this series. Blaud's pills were given daily. The experimental histories give the complete list of anemia experiments on any given dog and are referred to in each experiment. The control experiments are frequently given in the other papers of this series, but the proper reference is appended to the experiment dealing with iron or hemoglobin. We hope to report experiments in the near future dealing with other drugs used in the treatment of anemia. We expect to test many other pigment substances besides hemoglobin as to their influence on blood regeneration. This includes animal and vegetable pigments as they occur in various meats, fish and vegetables.

Table 68 represents a long anemia experiment with Blaud's pills which is conclusive in showing the complete failure of this drug (carbonate of iron) to influence blood regeneration under the conditions of the experiment. Subsequent experiments will make it clear that *Blaud's pills are inert* in so far as any influence on this type of anemia is concerned.

The experimental history of this dog (table 18–b) gives the reaction of this animal to other diet factors and establishes the type reaction to secondary anemia. It is fair to say that this same type of blood regeneration would be expected without the influence of Blaud's pills. This dog is more resistant than usual and tolerated this limited diet for a long period without any symptoms of dietary deficiency disease. The falling hemoglobin, red cell count and weight curves, however, may indicate an impending dietary deficiency complex which did not develop because of the change to a liberal mixed diet.

The Blaud's pills were given daily and crushed before administration by mouth. Sufficient bread and skim milk were given to maintain the body weight constant until the last 2 weeks. During the first 8 weeks the red cell hematocrit is stationary but the hemoglobin rises slowly. During this period the red count rises slowly and uniformly

TABLE 68

Blood regeneration—bread, milk and Blaud's pills. Dog 16–160. Bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
2/12	939	978	489	489	50	96	0.73	6,6	6,2	7.70	127	
2/12	Diet: Bread and milk											
2/13	Bled 244 cc.											
2/14	Bled 244 cc.											
2/15	267	580	423	157	27	46	0.77	3,0	6,8	7.40	80	
2/15	Bread, milk and 2 Blaud's pills daily											
2/21	348	809	566	243	30	43	0.86	2,5	10,8	7.50	108	
2/28	375	694	493	201	29	54	0.71	3,8	7,4	7.40	94	
3/7	393	667	487	180	27	59	0.69	4,3	5,6	7.30	91	*
3/14	428	738	524	214	29	58	0.66	4,4	11,0	7.20	104	*
3/21	335	697	495	202	29	48	0.52	4,6	7,4	6.80	103	*
3/28	312	625	431	194	31	50	0.48	5,2	10,0	7.00	89	*
4/5	382	694	486	208	30	55	0.48	5,7	6,8	6.80	102	*
4/11	368	681	470	211	31	54	0.46	5,9	13,6	7.00	97	*
4/18	500	725	471	254	35	69	0.51	6,7	11,2	7.30	99	*
4/25	576	728	480	258	35	78	0.51	7,7	5,6	7.20	102	*
5/4	560	700	455	245	35	80	0.52	7,7	8,6	7.00	100	*
5/11	617	771	455	316	41	80	0.53	7,6	12,4	7.40	104	*
5/18	624	762	457	305	40	82	0.55	7,4	9,4	7.20	106	*
5/25	688	819	467	352	43	84	0.57	7,3	6,6	7.00	117	*
6/1	716	721	418	303	42	99	0.59	8,4	6,6	6.80	106	*
6/8	830	847	491	355	42	98	0.64	7,6	5,4	7.00	121	*
6/15	833	833	500	333	40	100	0.66	7,6	5,8	6.50	127	*
6/27	705	839	520	319	38	84	0.62	6,8	10,0	6.50	129	*
7/11	477	597	394	203	34	80	0.67	6,0	7,6	6.20	96	*
7/16	456	570	393	177	31	80	0.74	5,4	6,2	5.90	97	*

* Marked fragmentation of red blood cells.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.
Experimental history, see table 18–b.

and continues this rise to a figure even above normal. It is noted, however, that there was marked fragmentation of the red cells and we can scarcely account for this great increase in red cells (2,500,000 to 8,400,000) with the red cell hematocrit showing only a rise from 27 per cent to 42 per cent, except on the ground of red cell fragmentation or abortive red cell construction. The hemoglobin does not keep pace with the red count and a fall in the color index from 77 per cent to 46 per cent is recorded.

TABLE 69

Blood regeneration—bread, milk and Blaud's pills. Dog 17-193. Bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
4/24	1089	1100	517	584	53	99	0.75	6,6	15,8	8.40	131	
4/24	Diet: Bread and milk											
4/25	Bled 275 cc.											
4/26	Bled 275 cc.											
4/27	333	710	547	163	23	47	0.87	2,7	24,0	8.20	87	
4/27	Diet: Bread, milk and 2 Blaud's pills daily											
5/2	458	683	492	191	28	67	0.82	4,1	9,4	8.00	85	
5/9	636	795	493	302	38	80	0.75	5,3	9,4	8.00	99	
5/16	856	1006	533	473	47	85	0.76	5,6	6,6	8.00	102	
5/23	1008	1039	561	478	46	97	0.69	7,0	7,4	8.00	129	*
5/30	1041	1021	521	500	49	102	0.67	7,6	12,4	8.20	124	*
6/6	1055	1014	527	487	48	104	0.65	8,0	6,2	7.90	128	*

* Fragmentation of red blood cells.

No previous anemia experiments on this dog.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

The high water mark for blood regeneration is noted after 3½ months and the level at this time is far from normal. Subsequently there is a loss in red cell hematocrit, red count, hemoglobin and pigment volume. We believe this indicated a tendency toward a dietary deficiency disease which would have developed had the bread and milk diet been continued. The plasma volume as usual is constant throughout the entire experiment with the exception of the last 2 weeks.

The next experiment (table 69) shows a fairly complete blood regeneration during a period of 5 weeks on a bread and milk diet plus Blaud's pills. It is to be observed that no previous anemia experiments had been done on this dog and the capacity for blood regeneration may be greater under such circumstances. The reaction is not unusual, however, in view of the liberal amounts of bread and milk which were sufficient to maintain the body weight. It cannot be granted that the Blaud's pills had any influence upon the blood regeneration.

TABLE 70

Blood regeneration—crackermeal, lard, butter, milk powder and Blaud's pills. Dog 17-205. Bull mongrel, male, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/4	1182	1055	538	518	49	112	0.70	8,0	10,8	12.60	84	
3/4	Diet: 279 grams crackermeal, 10 grams lard, 10 grams butter											
3/6	Bled 274 cc.											
3/7	Bled 254 cc.											
3/9	605	840	605	235	28	72	0.82	4,4	30,4	12.1	69	*Anis.
3/9	Diet: 142 grams crackermeal, 10 grams lard, 10 grams butter, 279 grams milk powder, 2 Blaud's pills daily											
3/15	565	807	573	234	29	70	0.88	4,0	27,4	11.40	71	*Anis.
3/20	798	928	622	306	33	86	0.93	4,6	9,2	11.20	83	*Anis.
3/27	682	802	545	256	32	85	0.79	5,4	19,2	10.90	73	*Anis.++
4/3	983	919	597	323	35	107	0.82	6,5	21,6	10.90	84	*Anis.++
4/9	795	750	480	270	36	106	0.79	6,7	7,4	9.60	78	*Anis.++
4/9	Dietary deficiency disease. Recovery.											

* Anisocytosis of red blood cells.

Refer to table 34 for control.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Table 70 is of considerable interest because we are able to refer to a control period (table 34) on a similar diet but without the Blaud's pills. If anything the control period shows slightly more blood regeneration during the first 5 weeks. The control experiment was of much longer duration (12 weeks) without dietary deficiency disease symptoms. This may be due to the fact that this control experiment (table 34) on crackermeal, lard and butter was the first anemia experiment done on this animal.

TABLE 71

Blood regeneration—crackermeal, lard, butter and Blaud's pills. Dog 17–157. Coach mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/4	1084	976	537	439	45	111	0.82	6,8	8,4	9.80	99	
3/4	Diet: 199 grams crackermeal, 10 grams lard, 10 grams butter											
3/6	Bled 254 cc.											
3/7	Bled 234 cc.											
3/9	614	818	597	221	27	75	1.00	3,7	10,2	9.10	90	
3/9	Diet: 201 grams crackermeal, 10 grams lard, 10 grams butter, 2 Blaud's pills daily											
3/15	756	869	600	269	31	87	0.99	4,4	14,8	9.00	97	*
3/20	801	843	531	312	37	95	1.00	4,7	11,4	8.50	99	*
3/27	848	848	517	331	39	100	0.89	5,6	8,2	8.40	101	*
4/3	943	865	519	346	40	109	0.88	6,2	18,8	8.30	104	*
4/9	984	878	536	342	39	112	0.85	6,6	11,4	8.30	106	*

* Slight anisocytosis of red blood cells.

Experimental history, see table 66–b.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

The control without Blaud's pills and this experiment (table 70) with Blaud's pills show practically identical anemia figures for red cell hematocrit and hemoglobin. The amount of red cell and hemoglobin regeneration is practically identical in the 5 weeks in the two experiments. This again gives evidence that Blaud's pills are inert under these controlled conditions of experimental anemia and regeneration.

Table 71 shows a little more blood regeneration than usual but not enough to be able to attribute any of the reaction to the Blaud's pills. The red cell hematocrit reads 39 per cent at the end of 1 month and this is not a normal figure. The hemoglobin was low to start with and returns to this level in 4 weeks. The type reaction of this dog is well established by the list of other anemia experiments given in table 66–b.

TABLE 72

Blood regeneration—bread, milk and Blaud's pills—splenectomy. Dog 17–142. Coach mongrel, female, age 4 to 5 months

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
4/24	842	772	386	386	50	109	0.91	6,0	19,8	8.00	96
4/24	Diet: Bread and milk										
4/25 4/26	Bled 193 cc. Bled 193 cc.										
4/27	323	609	457	152	25	53	0.98	2,7	20,2	8.00	76
4/27	Diet: Bread, milk and 2 Blaud's pills daily										
5/2	390	600	390	210	35	65	0.90	3,6	18,4	7.80	76
5/9	760	835	451	384	46	91	0.83	5,5	13,6	7.90	106
5/16	837	790	411	379	48	106	0.80	6,6	7,2	7.70	103
5/23	997	906	453	453	50	110	0.75	7,3	6,4	7.70	117
5/30	890	832	416	416	50	107	0.70	7,7	11,4	8.00	104
6/6	950	863	440	423	49	110	0.73	7,5	11,6	7.70	112

No previous anemia experiments on this dog.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Table 72 shows a reaction to the first anemia experiment which has been noted in other experiments. It is not the rule but is frequent enough so that we must always be on our guard in discussing the *first* anemia experiment on any given dog. There may be this remarkable reserve which enables the dog to give an unusual blood regeneration even on a most unfavorable diet. The blood regeneration is complete in 3 to 4 weeks and thereafter is maintained for the next 3 weeks at the normal level. Refer to tables 74 and 75 for meat diet controls with no previous bleeding. The fact that the dog was very young (4 to 5 months) and was without a spleen is thought to be without influence on this general reaction noted in other dogs (adult and non-

TABLE 73

Blood regeneration—bread, milk and Blaud's pills—splenectomy. Dog 17-37. Bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
3/26	927	850	459	391	46	109	0.80	6,8	9,4	10.60	80	
3/29	Bled 212 cc. and 212 cc. (refer to table 8)											
4/23	Diet: Bread and milk following fasting experiment of 3 weeks' duration											
4/23	540	772	502	270	35	70	0.66	5,3	7,8	6.90	112	
4/30	578	802	521	281	35	72	0.67	5,4	11,6	8.20	98	
5/7	566	808	517	291	36	70	0.71	4,9	7,4	8.40	96	
5/14	662	808	517	291	36	82	0.68	6,0	6,8	8.40	96	*
5/21	656	830	531	299	36	79	0.69	5,7	5,6	8.90	93	
5/28	566	833	553	283	34	68	0.68	5,0	7,0	8.30	100	
6/4	524	759	530	228	30	69	0.73	4,7	5,2	8.60	88	
6/11	528	754	529	226	30	70	0.78	4,5	14,6	8.60	88	
6/11	Diet: Bread, milk and 2 Blaud's pills daily											
6/18	631	809	517	291	36	78	0.62	6,3	6,4	8.40	96	†
6/25	514	858	626	231	27	60	0.79	3,8	53,0	8.30	103	†
7/2	439	708	517	191	27	62	0.79	3,9	18,2	8.40	84	†
7/9	537	790	521	268	34	68	0.76	4,5	13,0	8.60	92	†
7/16	648	762	480	282	37	85	0.77	5,5	9,3	8.40	91	†

* Red cells are small and much fragmented.

† Red cells are large and fairly uniform in size.

Experimental history, see table 8-b.

Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

splenectomized). We have no reason to suspect that the Blaud's pills were concerned in this reaction which has been noted in control experiments on the same diet without the Blaud's pills.

The second splenectomy experiment (table 73) shows a negative reaction with bread and milk alone as well as with bread and milk plus Blaud's pills. There are peculiar fluctuations during certain weeks in both these periods and we believe these ups and downs are referable to the splenectomy. It is significant that the hemoglobin and red cell hematocrit changes are not associated with any constant change in plasma volume. The red count fluctuates with the pigment curve and we must assume periodic constructive or destructive waves influencing the red cells in the blood stream. From data already published (1) dealing with bile excretion in splenectomized dogs with simple

TABLE 74

Blood regeneration—meat and Blaud's pills. Dog 17-191. Bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
4/24	1352	1206	567	640	53	112	0.71	7,9	13.4	9.50	127
4/24	Diet: Bread and milk										
4/25	Bled 301 cc.										
4/26	Bled 301 cc.										
4/27	382	780	600	187	24	49	0.79	3,1	17,6	9.30	84
4/27	Diet: Meat and 2 Blaud's pills daily										
5/2	536	800	543	264	33	67	0.91	3,7	17,8	9.50	84
5/9	1030	1064	585	479	45	97	0.90	5,4	8,2	9.50	112
5/16	1282	1256	653	604	48	102	0.73	7,0	11,8	9.80	128
5/23	1530	1377	647	730	53	111	0.66	8,4	8,8	9.70	142
5/30	1571	1366	615	752	55	115	0.65	8,8	18,6	10.00	136
6/6	1510	1314	591	419	55	115	0.66	8,7	7,2	9.60	137

No previous anemia experiments on this dog.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

anemia we may suspect that blood destruction may be in part responsible for these irregularities in the level of the curves of pigment volume, red cell and hemoglobin values. The color index shows no change. There is a note to the effect that the red cells are larger and more uniform in size during the period of iron feeding. A single observation of this nature is of interest but does not call for discussion at this time.

TABLE 75

Blood regeneration—meat and Blaud's pills—splenectomy. Dog 17-163. Bull mongrel, male, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
4/24	918	918	450	468	51	100	0.75	6,7	14,4	9.00	102
4/24	Diet: Bread and milk										
4/25	Bled 229 cc.										
4/26	Bled 229 cc.										
4/27	340	679	509	170	25	50	1.00	2,5	17,6	8.90	76
4/27	Diet: Meat and 2 Blaud's pills daily										
5/2	538	803	514	289	36	67	0.90	3,7	19,2	9.00	89
5/9	790	909	509	400	44	87	0.78	5,6	19,6	8.90	102
5/16	924	880	475	405	46	105	0.78	6,7	8,2	8.90	98
5/23	1140	1096	559	538	49	104	0.75	6,9	12,4	8.80	124
5/30	907	863	423	440	51	105	0.63	8,3	9,2	9.00	96
6/6	1080	1002	491	520	51	106	0.65	8,2	13,8	8.60	117

No previous anemia experiments on this dog.
Experimental history, see table 38-b.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

The two meat feeding experiments (tables 74 and 75) give the expected reaction on this diet. The splenectomy dog reacts the same as does the normal dog. Sufficient meat is given to maintain a constant body weight. Both these dogs had not been used previously for anemia experiments; refer to table 72 which illustrates the reserve capacity sometimes exhibited by such dogs. We have no reason to suppose that the Blaud's pills were in any way concerned in this reaction.

Hemoglobin experiments.—Table 76 gives an experiment of much interest and we are able to submit the control experiment (table 16). This same dog a few months previously was made anemic and placed upon a sugar diet. After a period of 4 weeks the level of pigment volume, hemoglobin and red cell hematocrit was practically the same as that observed immediately after the bleeding. The total blood regeneration then in the control experiment was zero. There was a trifling gain in the red count.

TABLE 76

Blood regeneration—hemoglobin and sugar—metabolism. Dog 17-28. Bull mongrel, female, young adult

DATE, 1917	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
5/7	1895	1709	769	940	55	111	0.66	8,4	13,2	12.50	136	
5/7	Diet: Mixed food											
5/8	Bled 427 cc.											
5/9	Bled 427 cc.											
5/11	594	914	640	274	30	65	0.76	4,3	22,0	12.20	76	
5/11	Diet: 50 grams cane sugar, 25 grams glucose, 10 grams washed red blood cells											
5/14	558	979	676	303	31	57	0.75	3,8	11,2	11.50	85	
5/21	676	1055	665	390	37	64	0.70	4,6	6,8	10.80	98	*
5/28	856	1141	673	468	41	75	0.71	5,3	9,6	10.10	113	*
6/4	887	1137	603	534	47	78	0.58	6,7	9,6	9.50	119	*
6/11	920	1180	672	508	43	78	0.59	6,6	8,0	8.90	133	*
6/18	1085	1277	664	612	48	85	0.50	8,5	10,0	8.30	154	*

* Marked fragmentation of red cells.
400 cc. water given by stomach tube daily.
Experimental history, see table 6-b; see table 16 for control.
Blood volume with dry oxalate. Hemoglobin by Sahli tubes.

Under identical conditions on a sugar diet plus 10 grams washed, dried red blood cells we see a very different reaction (table 76). The

TABLE 76-A

Total urinary nitrogen—hemoglobin and sugar. Dog 17-28

DATE, 1917	TOTAL NITROGEN 24 HOURS URINE	URINE TOTAL 24 HOURS	WEIGHT	REMARKS
	grams	*cc.*	*pounds*	
5/11	3.55	490	26.4	0 feces
5/12	3.19	480	25.8	Solid black stool
5/13		361	25.5	Solid black stool
5/14	3.50	386	25.3	0 feces
5/15	2.52	485	25.0	Slight diarrhea
5/16	2.58	358	24.7	Solid feces
5/17	2.38	390	24.5	0 feces
5/18	2.35	480	24.4	Trace of feces
5/19	2.30	403	24.1	Slight diarrhea
5/20	2.46	410	24.0	0 feces
5/21	2.46	420	23.8	0 feces
5/22	2.63	465	23.4	0 feces
5/23	2.41	383	23.4	0 feces
5/24	2.94	400	23.0	Diarrhea +
5/25	2.74	376	22.7	Diarrhea +
5/26	2.66	412	22.6	0 feces
5/27	2.52	395	22.4	0 feces
5/28	2.35	424	22.2	0 feces
5/29	2.46	370	21.9	0 feces
5/30	2.63	410	21.8	0 feces
5/31	2.13	510	21.6	0 feces
6/1	2.32	405	21.4	0 feces
6/2	2.18	405	21.4	0 feces
6/3	2.30	425	21.1	0 feces
6/4	2.24	426	20.8	
6/5	2.44	425	20.8	0 feces
6/6	3.00	417	20.5	Formed feces
6/7	2.24	373	20.2	Soft feces
6/8	2.46	410	20.0	Solid feces
6/9	2.58	419	19.9	0 feces
6/10	2.60	425	19.8	0 feces
6/11	2.63	407	19.5	0 feces
6/12	2.83	405	19.5	0 feces
6/13	2.83	375	19.4	0 feces
6/14	2.88	490	19.2	0 feces
6/15	3.00	480	18.9	0 feces
6/16	3.28	411	18.6	Solid feces
6/17	3.42	395	18.4	0 feces
6/18	3.02	400	18.3	

initial anemia level in the two experiments is practically identical, also the body weight, normal initial blood pigment, etc. The only point in which these two experiments differ lies in the 10 grams of red cells added to the sugar diet. At the end of 4 weeks the control shows a gain of zero in pigment substance but the red cell feeding gives a substantial gain of 13 per cent hemoglobin, 17 per cent red cell hematocrit and 300 units pigment volume. There is a gain of 2,400,000 in the red cell count. The subsequent 2 weeks show a distinct gain over the level just noted. This is in notable contrast to the expected reaction on a sugar diet.

The urinary nitrogen and fluid excretion figures are given (table 76–a) and show the expected values. During the last week of the experiment there is a distinct rise in the nitrogen output. We have come to look upon this as an early sign of intoxication which if not heeded may be followed by severe clinical disturbances and death. This dog promptly recovered when placed on a mixed diet.

It is to be noted that *whole red cells* were used in this experiment, that is, hemoglobin plus red cell stroma. In the following experiments hemoglobin alone was used. We might point out the difference in the color index observed in these two conditions but feel that the data are not sufficient to establish this very interesting point. We are gradually collecting data which indicate the conditions most favorable for stroma production and these experiments will be presented at another time.

The next hemoglobin experiment (table 77) is not very convincing as we do not have a suitable control of the bread and milk factors in this dog. Moreover this is the first anemia experiment on this dog and under such circumstances this reaction is often atypical, as has been noted before. We may say that the blood regeneration due to the bread and milk plus hemoglobin might be identical with a reaction on bread and milk alone. We cannot point with certainty to any difference which can be attributed to the hemoglobin. This experiment is unlike the others of this group which give positive evidence that hemoglobin does influence the curve of blood regeneration. Finally, we must conclude that this experiment does not give any evidence against the value of hemoglobin but it also gives no positive support to the other experiments.

Table 78 presents a long experiment in which hemoglobin is given by mouth during one period and by intravenous injection during a subsequent period. We feel that there is good evidence that hemoglobin did influence favorably the blood regeneration. The anemia level produced by three bleedings was slightly below the average. The first period of sugar feeding plus 10 grams of hemoglobin by mouth shows a notable gain during 3 weeks. The hemoglobin rises 50 per cent and the pigment volume from 433 to 810. There is a corresponding rise in the red cell hematocrit and red cell count. On sugar alone we recall that the gain in these pigment factors is only trifling, perhaps 10 per cent in hemoglobin and corresponding amounts in the other readings. We must hold the hemoglobin responsible at least for a part of this favorable reaction.

TABLE 77

Blood regeneration—powdered hemoglobin and bread and milk. Dog 18–124. Terrier mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*
5/16		515	273	234	45.5					5.80	89
5/26	Diet: Bread and milk										
5/27	488	548	302	241	43.8	89	0.53	8,4	10,4	6.10	90
5/28	Bled 137 cc.										
5/29	Bled 137 cc. No distress										
5/29	Diet: 125 grams bread (ground and dried), 300 cc. milk, 5 grams hemoglobin*										
5/31	207	398	308	87	21.8	52	0.59	4,4	12,8	5.70	70
6/5	370	481	348	129	26.9	77	0.80	4,8	11,2	5.75	84
6/14	376	522	357	160	30.6	72	0.61	5,9	8,6	5.35	98
6/21	421	513	325	183	35.7	82	0.60	6,8	7,8	5.20	99
6/26	342	417	266	145	34.7	82	0.66	6,2	7,2	5.25	79

* Hemoglobin: Defibrinated blood centrifuged, cells washed twice with N/1 salt solution, 2 volumes distilled water added, allowed to lake over night, centrifugalized, stroma removed, hemoglobin dried and powdered.

No previous anemia experiments on this dog.

TABLE 78

Blood regeneration—sugar and hemoglobin—crackermeal, milk and hemoglobin. Dog 17-157. Coach mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	cc.	cc.	per cent	per cent				kgm.	cc.	
8/28	1366	1027	518	504	49.0	133	1.00	6,7	14,4	10.10	102	*Slight
8/28	Diet: Crackermeal and milk											
8/29	Bled 257 cc.											
8/30	Bled 257 cc.											
8/31	565	777	560	215	27.6	73				9.65	81	
8/31	Bled 194 cc.											
9/3	433	731	544	179	24.5	59	0.98	3,0	13,8	9.25	79	*Poik.+
9/10	Diet: 75 grams sugar, 25 grams dextrose, 10 grams hemoglobin† by stomach tube											
9/10	646	848	590	249	29.4	76	0.84	4,5	9,4	8.40	100	*Poik.+ Vomited
9/16	750	896	593	294	32.8	84	0.82	5,1	6,8	8.00	113	* Poik.+
9/25	810	788	492	293	37.2	102	0.86	5,9	6,6	7.25	109	* Poik.+
9/25	Diet: 100 grams sugar, 30 cc. hemoglobin intravenously‡											
9/30	625	742	463	271	36.5	84	0.70	6,0	6,2	6.95	106	* Poik.+
9/30	Diet: 200 grams crackermeal, 500 cc. milk, and hemoglobin intravenously‡											
10/11	662	752	465	280	37.2	88	0.76	5,8	13,6	7.55	100	
10/16	805	830	502	322	38.8	97	0.73	6,3	12,8	7.55	110	* Slight
10/16	Diet: 200 grams crackermeal, 500 cc. milk											
10/23	722	902	570	322	35.8	80	0.67	6,0	16,0	8.00	112	* Poik.+

* Poikilocytosis of red blood cells.

† Blood centrifugalized, washed once with salt solution; 2 volumes of distilled water added to washed, packed red blood cells. Allowed to stand 24 hours. Centrifugalized and stroma removed. Dried and powdered.

‡ For injection into the vein: With aseptic technique blood is centrifugalized, washed once with salt solution; 20 cc. distilled water added to 10 cc. washed, packed red blood cells. Allowed to stand 4 hours, added 2.5 cc. of 10 per cent salt solution. Centrifugalized and stroma removed. Total amount injected intravenously daily.

Experimental history, see table 66-b.

When the sugar diet is continued but the hemoglobin is given intravenously, we note a fall in hemoglobin and pigment volume but no change in the red cell hematocrit, red cell count and plasma volume. We have no good explanation to fit these observed facts.

TABLE 79

Blood regeneration—hemoglobin injection intravenously. Dog 17-27. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/9	1914	1454	718	727	50.0	133	0.75	8,7	12,0	16.15	90	
8/9	Diet: Bread and milk											
8/12 8/13	Bled 364 cc. No distress Bled 364 cc. No distress											
8/15	749	1040	732	297	28.6	72				15.55	67	
8/15	Bled 260 cc.											
8/17	817	1034	718	311	30.1	79	0.95	4,1	24,6	15.45	67	* Poik.++
8/17	Diet: Hemoglobin injection intravenously;† 75 grams sugar, 25 grams dextrose, 200 cc. water daily by stomach tube											
8/23	908	1080	710	370	34.3	84	0.76	5,5	14,8	14.55	74	
8/30	1155	1100	647	442	40.2	105	0.75	7,0	5,6	13.75	80	* Poik.++
9/2	Accidental death											

* Poikilocytosis of red blood cells.

† Hemoglobin: Blood drawn from normal dog with aseptic precautions. Centrifugalized, washed once with salt solution; 20 cc. distilled water added to 10 cc. washed, packed red blood cells. Allowed to stand 4 hours, added 2.5 cc. of 10 per cent salt solution. Centrifugalized and stroma removed. Total amount injected intravenously daily.

Experimental history, see table 15-b; see sugar control, table 15.

The 2 weeks following on a crackermeal and milk diet plus hemoglobin intravenously show a slight gain in pigment substance. Even this slight gain may have some significance when we recall that it occurred following a long period of limited diet intake. Hemoglobin regeneration under such unfavorable circumstances is very difficult and becomes increasingly difficult as the limited diet periods are extended.

Table 79 gives an experiment which was unfortunately terminated at the end of 2 weeks by an accident. We are able to refer to a control reaction on sugar feeding alone (table 15). This table shows a slight gain in hemoglobin, red cell hematocrit and pigment volume during the first week on the sugar diet. The second week shows no gain. Compare with this reaction the figures in table 79 which show a gain in the first week which may be compared with the control but the second week intead of the stationary level in the control shows a distinct gain in red cells, pigment volume, hemoglobin, etc. We feel that a part of this gain is to be explained by the hemoglobin injections.

Table 80 illustrates another type of experiment in which the hemoglobin injections are given under conditions very unfavorable for blood regeneration. There is a 3-week period of sugar feeding during which time there is zero gain in pigment substance. There is a slight but distinct gain following 5 days of hemoglobin injection and further slight gains on a crackermeal and milk diet with daily hemoglobin injections. Some of the subsequent gains in hemoglobin, red cell hematocrit and red cells may be due in part to the hemoglobin injections which in all probability cannot be at once utilized. The crackermeal and milk alone or with yeast can account for very little blood regeneration when given subsequent to a long period of sugar feeding. The evidence for the favorable influence of hemoglobin injections is not as strong as in some of the other experiments which have the complete control periods.

The last hemoglobin experiment (table 81) is to be compared with table 76. The hemoglobin in this instance is given intraperitoneally so that the absorption might be rapid and the elimination of slight amounts through the urine be obviated. The influence of a different set of phagocytic cells might well be a factor but the gross results are much the same as regards blood regeneration.

Three weeks of sugar feeding plus hemoglobin injection give an amount of blood regeneration which cannot be explained as due to the sugar feeding. We note a rise of hemoglobin from 72 to 120 per cent and red cell hematocrit from 27 to 45 per cent. The control sugar diet figures would show only trifling gains. Subsequent weeks on a crackermeal and milk diet do not show much gain except in the red count. The final period of mixed diet as usual brings the dog back to a high normal figure.

TABLE 80

Blood regeneration—hemoglobin intravenously. Dog 16-160. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb, PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/28	1835	1103	456	654	58.2	166	1.10	7,6	9,6	10.15	109	
8/28	Diet: Bread and milk											
8/29	Bled 276 cc.											
8/30	Bled 276 cc.											
9/1	648	771	524	243	31.5	84				9.55	81	*
9/1	Bled 193 cc.											
9/3	448	696	513	177	25.5	64	0.80	4,0	15,4	9.50	73	* Poik.++
9/3-9/26	Diet: 75 grams cane sugar, 25 grams dextrose, 200 cc. water by stomach tube (table 18)											
9/25	429	664	474	182	27.4	64	0.61	5,2	9,6	7.35	90	* Poik.++
9/26	Diet: Hemoglobin intravenously,‡ 30 cc., and 100 grams sugar by stomach tube											
9/30	437	618	421	194	31.4	71	0.68	5,2	12,4	7.15	86	* Poik.++†
9/30	Diet: Hemoglobin intravenously,‡ 30 cc., and 200 grams crackermeal, 500 cc. milk											
10/11	506	712	477	227	31.9	71	0.58	6,1	11,0	7.75	92	* Poik.++
10/16	586	740	495	237	32.0	79	0.65	6,1	5,0	7.80	95	* Poik.++†
10/16	Diet: Hemoglobin injection discontinued; 200 grams crackermeal, 500 cc. milk											
10/23	670	826	536	281	34.0	81	0.65	6,2	8,4	8.05	103	* Poik.++†
10/23	Diet: 1 gram dried brewer's yeast, 200 grams crackermeal, 500 cc. milk.											
10/30	590	808	536	264	32.7	73	0.47	7,7	15,2	8.30	97	* Poik.++†
11/6	720	868	547	317	36.5	83	0.66	6,3	8,6	8.20	106	* Poik.+
11/12	784	883	532	342	38.7	89	0.50	8,9	7,2	8.25	107	* Poik.+
11/13	Diet: Mixed diet											
11/22	726	853	503	341	40.0	85	0.42	10,2	13,6	8.80	97	* Poik.+
11/29	1122	1020	547	463	45.4	110	0.69	8,0	19,0	9.25	105	Excellent condition

* Poikilocytosis of red blood cells.

† Shadow cells.

‡ Hemoglobin: Blood taken with aseptic precautions, centrifugalized, washed once with salt solution; 20 cc. of distilled water added to 10 cc. washed, packed red blood cells. Allowed to stand 4 hours, added 2.5 cc. of 10 per cent salt solution. Centrifugalized and stroma removed. Total amount injected intravenously daily.

Experimental history, see table 18-b.

TABLE 81

Blood regeneration—hemoglobin intraperitoneally. Dog 17-28. Bull mongrel, female, young adult

DATE, 1918	PIGMENT VOLUME = Hb. PER CENT TIMES BLOOD VOLUME	BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	R. B. C. HEMATOCRIT	Hb.	COLOR INDEX	R. B. C. MILLION	W. B. C.	WEIGHT	BLOOD PER KILOGRAM	REMARKS
		cc.	*cc.*	*cc.*	*per cent*	*per cent*				*kgm.*	*cc.*	
8/9	2417	1580	680	887	56.1	153	0.84	9,1	14,2	17.00	93	
8/9	Diet: Bread and milk											
8/12	Bled 395 cc. No distress											
8/13	Bled 395 cc. Diet: Crackermeal and milk											
8/15	1015	1142	750	387	33.8	89				16.30	71	
8/15	Bled 286 cc.											
8/17	756	1050	758	281	26.8	72	0.97	3,7	23,6	15.90	66	* Poik.+
8/19	Hemoglobin† (intraperitoneal injection). Diet: 75 grams sugar, 25 grams dextrose, water											
8/23	794	1030	708	310	30.1	77	0.69	5,6	21,4	15.40	67	* Poik.
8/30	1133	1193	685	430	36.0	95	0.76	6,2	12,6	14.55	82	* Poik.+
9/4	Hemoglobin† (intraperitoneal injection). Diet: 100 grams sugar in 500 cc. water											
9/6	1670	1385	755	608	45.0	120	0.83	7,2	11,0	13.40	103	* Poik.++
9/6	Hemoglobin discontinued. Diet: 200 grams crackermeal, 500 cc. milk kaolin											
9/13	1550	1450	876	566	39.2	107	0.68	7,9	9,8	14.00	103	
9/19	1433	1310	752	545	41.6	109	0.71	7,7	12,2	13.90	94	* Poik.
9/27	1253	1130	658	468	41.4	111	0.71	7,8	10,6	14.30	79	* Poik.
10/9	1635	1363	668	682	50.5	120	0.77	7,8	12,2	14.20	96	
10/18	1836	1386	680	694	50.0	132	0.66	10,1	9,0	14.45	96	
10/25	1550	1352	704	636	47.0	115	0.64	9,0	10,8	14.60	93	
10/25	Diet: 200 grams crackermeal, 500 cc. milk, 1 gram dried, powdered brewer's yeast, kaolin											
10/31	1415	1400	750	638	45.4	101	0.65	7,8	11,8	15.00	93	
10/31	Diet: Mixed diet											
11/7	1975	1555	710	829	53.3	127	0.74	8,6	10,8	15.10	103	
11/13	2000	1600	743	840	52.5	125	0.63	9,9	20,2	15.05	106	

* Poikilocytosis of red blood cells.

† Hemoglobin: 10 cc. sterile, washed, packed red blood cells and 20 cc. distilled water. Centrifugalized and stroma removed. Intraperitoneal injection. Experimental history, see table 6-b; see table 76 for hemoglobin feeding.

SUMMARY

Blaud's pills are inert when added to various diets which do or do not favor rapid blood regeneration. We may not assume without positive proof that inorganic iron is of value in the treatment of secondary anemia.

Splenectomy may or may not modify this blood regeneration reaction. Limited diets following anemia periods associated with splenectomy may be the cause of fluctuations in the normal expected curve of blood regeneration.

Hemoglobin (by mouth, intravenously or intraperitoneally) exerts a distinctly favorable influence upon subsequent blood regeneration.

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PHYSIOLOGIC CHANGES PRODUCED BY VARIATIONS IN LUNG DISTENTION

III. IMPAIRMENT OF THE CORONARY CIRCULATION OF THE RIGHT VENTRICLE

RALPH HOPKINS AND FELIX P. CHILLINGWORTH

From the Physiological Laboratory, Tulane University of Louisiana, New Orleans

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In a previous communication a method was described by which the maximum blood pressure obtainable in the pulmonary arteries could be measured by the increase in intrapulmonic air pressure sufficient to prevent the passage of blood through the capillaries of the lungs (1). The mechanical blocking of the pulmonary circulation produced under these conditions was found to cause a maximum pulmonary arterial pressure of 85 mm. of mercury and a coincident complete disappearance of carotid pressure. In the present communication we record the results of some experiments to determine the critical point during progressive increase of intrapulmonic air pressure at which circulation of blood through the coronary arteries of the right ventricle becomes impossible because of the maintenance of a level of blood pressure in the pulmonary arterial and right cardiac systems equal to or higher than that of the general systemic circulation. Distention of the lungs to or beyond this critical point is almost immediately fatal and over-distention even when not attaining this critical degree also interferes with adequate oxygenation and nutrition of the heart. The necessity for a balance in favor of the diastolic systemic pressure over the diastolic right ventricular pressure has a bearing on artificial respiration effected by increase in the intrapulmonic air pressure. Loss of this balance through over-distention of the lungs may defeat the purpose for which artificial respiration is given by causing asphyxiation of the heart muscle even while the lungs are being over-ventilated. This observation is especially pertinent in the conditions in which, as is usually the case when artificial respiration is resorted to, the systemic arterial pressure is low and but little further fall is sufficient to reduce it below the level of pressure in the pulmonary arteries because the latter rises during great distention of the lungs while the systemic arterial pressure falls.

An endeavor is also made to show that even with the lungs distended in lesser degree than the critical point referred to, death may result owing to impairment of the coronary circulation of the right ventricle.

METHOD

The method employed to increase the pressure of the intrapulmonic air has been described in a previous communication (2). The use of the plethysmograph referred to facilitates the attainment of the degrees of pressure desired and obviates difficulties that would arise from forced expiratory efforts of the animals if the usual methods of intratracheal insufflation were employed. By placing the entire animal within the plethysmograph and connecting his trachea to the outside air, diminution of the pressure within the plethysmograph produces the same effects on the animal's relation to the air within his lungs as would result from an equal increase in the intrapulmonic air pressure while the exterior of the animal remained under atmopheric pressure. The decrease, therefore, in plethysmographic pressures is an exact measure of the actual increase of intrapulmonic air pressures and in referring to the effects of these pressures the terms are regarded as interchangeable.

A correction must be made in measuring blood pressures which are recorded during diminished plethysmographic pressures. To the blood pressures recorded in millimeters of mercury should be added the difference, in millimeters of mercury, existing between the atmospheric pressure and the pressure within the plethysmograph. The necessity for this correction is made obvious by the fact that with diminished plethysmographic pressures the carotid pressure may fall considerably below its zero line.

For recording blood pressures and the pressure within the plethysmograph mercury manometers were used and the readings in millimeters of mercury on the unreduced tracings have been multiplied by 2 when used in the subsequent paragraphs.

To record the pulmonary arterial pressure a branch of the left pulmonary artery was used and artificial respiration by intratracheal insufflation was performed after opening the chest and continued until the animal was placed in the plethysmograph. In the intervals between experiments, artificial respiration was maintained by rhythmical changes in the plethysmographic pressure.

Recovery after distention of the lungs is rapid and many experiments may be performed on one animal but the time factor as regards the duration of lung distention is of importance. In obtaining the results recorded in subsequent paragraphs no experiment exceeded one minute in duration.

RESULTS OF EXPERIMENTS

Four typical tracings are reproduced taken from a series of 25 experiments performed on 10 dogs, and a curve is plotted to show the effect of lung distention on the carotid and pulmonary arterial pressures. The tracings and the plot are first described and the results on the coronary circulation are subsequently discussed.

Figure 1. Synchronous records of the carotid and pulmonary arterial pressures are reproduced. The pressure within the plethysmograph is also shown. Air was exhausted from the plethysmograph during a period of one minute effecting a gradual fall in the intraplethysmographic pressure to 83 mm. of mercury below the atmospheric pressure. On the unreduced tracing from which this figure is reproduced the maximum fall in carotid pressure was equal to 212 mm. of mercury (actually 46 mm. below the carotid zero line), while the fall in pulmonary pressure was equal to 54 mm. of mercury (9 mm. below its zero line). The carotid pressure shows a corrected fall of 129 mm. (212–83), while the pulmonary pressure when corrected shows actually a rise of 29 mm. of mercury. When corrected, the minimum carotid and maximum pulmonary pressures are respectively 41 (preëxperimental pressure level 170 minus corrected fall 129) and 79 (preëxperimental level 50 plus rise resulting from correction 29). Respiratory waves, following a period of apnoea which was of 18 seconds' duration, are marked in both arterial curves but show only slightly in the plethysmographic record, since they were the result of spontaneous but ineffectual efforts on the part of the animal. It will be noted that during the last 14 seconds of the experiment the intraplethysmographic pressure rose slightly and that with this increase in pressure there occurred a rise in carotid while the pulmonary pressure continued to fall. It will also be noted that as the plethysmographic pressure is progressively lowered there is a gradual disappearance of pulse pressure in both the arterial curves. With sudden return to atmospheric pressure within the plethysmograph there is an almost

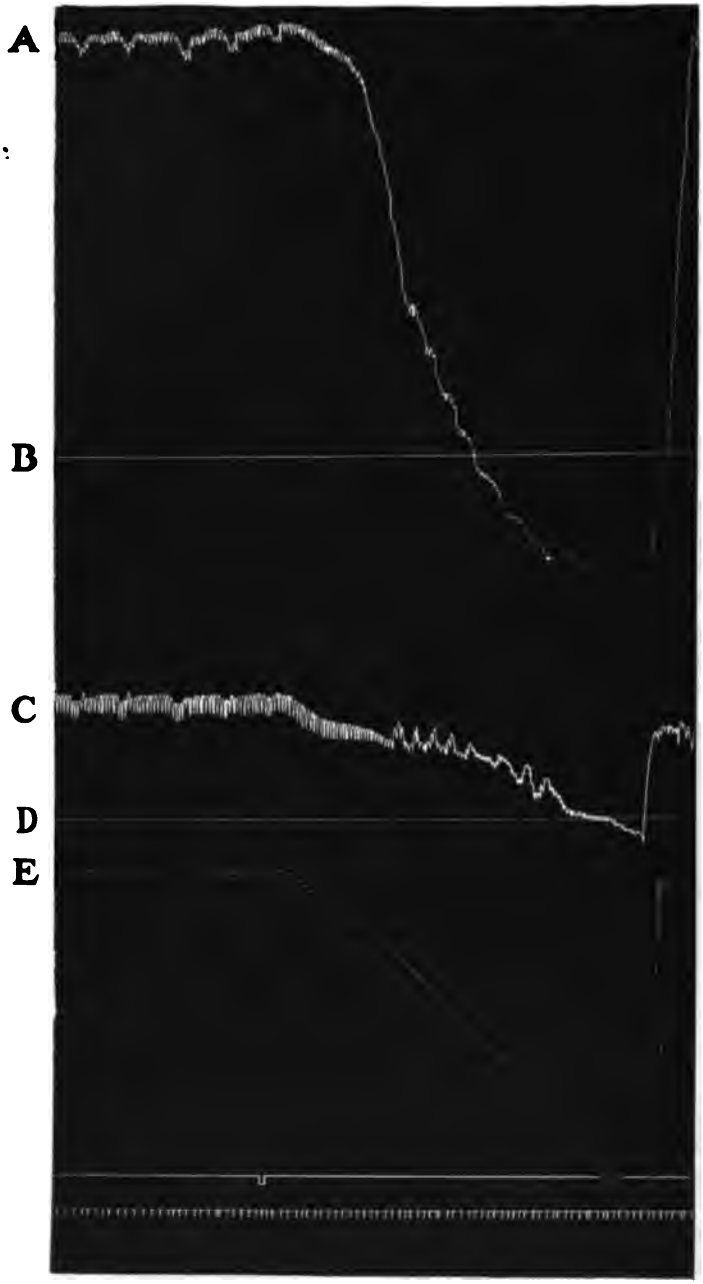

Fig. 1. Experiment of March 20, 1916. Dog 6 kilo. Ether. Time in seconds. *A*, Carotid blood pressure recorded by mercury manometer; *B*, Zero level for carotid blood pressure; *C*, Left pulmonary blood pressure recorded by mercury manometer; *D*, Zero level for pulmonary pressure; *E*, Plethysmographic pressure recorded by mercury manometer which is 68 mm. above the time record.

synchronous recovery of both arterial pressures to their preëxperimental pressure levels.

Figure 2, Records of carotid and pulmonary arterial pressures are reproduced from three parts of a typical tracing showing the effects of distention of the lungs in varying degree. The experimental procedure was varied in the experiment on this animal and the plethysmograph was not used. The lungs were inflated through the trachea which was connected to a reservoir containing air under pressure. The degree of pressure employed to distend the lungs is not recorded but

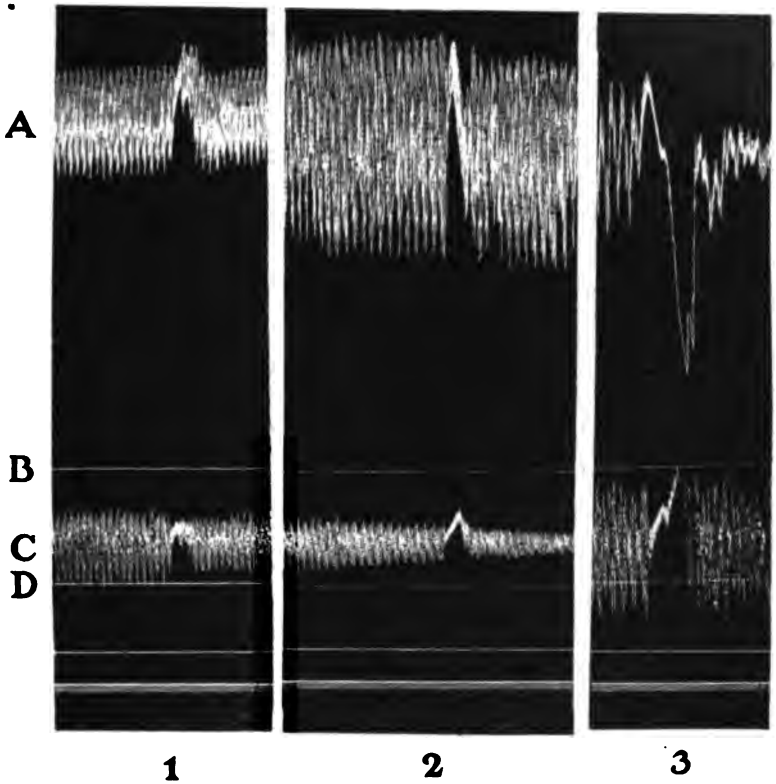

Fig. 2. Experiment of January 5, 1918. Dog 8.75 kilo. Ether. Time in seconds. *A*, Carotid blood pressure recorded by mercury manometer; *B*, Zero level for carotid blood pressure; *C*, Left pulmonary blood pressure recorded by mercury manometer; *D*, Zero level for pulmonary pressure. This level is 20 mm. above the time record. *1*, *2* and *3* show the progressive effects of increasing lung distention.

can be estimated from the effects produced upon the arterial pressures. Small caliber mercury manometers were used for recording blood pressures.

In the first of these tracings the lung distention is quite moderate and is accompanied by a slight rise in both arterial pressures. The systolic pulmonary pressure shows no rise but the mean blood pressure is higher due to maintenance of a higher diastolic level. Measurements of the unreduced tracing show a preëxperimental mean pulmonary pressure of 14 mm. of mercury which during inflation rises to 20.

In the second of this series the distention of the lungs is slightly greater and the mean pulmonary pressure rises from a preëxperimental level of 16 to 28 mm. of mercury. In this instance the systolic as well as the diastolic pressure rises during distention of the lungs, but the rise in the systolic is 8 mm. less than the rise in diastolic pressure.

In the third tracing distention of the lungs is in excess of that in either of the previous experiments and a corresponding increase is observed in the pulmonary arterial pressure which attains a maximum mean pressure of 43 mm. (29 mm. above the preëxperimental level of 14). Again it is noticeable that the rise in the diastolic exceeds that in the systolic pressure. A comparison of the mean pulmonary to the mean carotid pressures shows that, at the point of greatest lung distention, the pulmonary pressure exceeds that in the carotid artery by 6 mm. and also that the pulmonary diastolic pressure is slightly in excess of that of the carotid. The initial carotid pressure in this animal is 40 mm. lower than that plotted in figure 3, and as a result of this lower preëxperimental level a comparatively small fall in carotid pressure is sufficient to make the carotid equal to the pulmonary arterial pressure.

Figure 3. A curve is plotted from a typical tracing showing simultaneous carotid and pulmonary arterial pressures in millimeters of mercury during progressive increase of intrapulmonic air pressure. The broken line represents the former and the unbroken line the latter. The figures used have been corrected as explained in a previous paragraph. The ordinates indicate blood pressures and the abscissae the pressures in millimeters of mercury employed to distend the lungs.

The carotid pressure, while distention is progressing, shows an initial rise of 6 mm. of mercury attained when the excess of air pressure within the lungs is equal to 10 mm. of mercury. With increasing distention of the lungs the carotid pressure falls progressively, the maximum fall being attained with an increase in the intrapulmonic air pressure equal to 80 mm. of mercury, which was the greatest pressure used. With the exception of the small initial rise accompanying slight distention of the lungs, the fall in carotid pressure below the preëxperimental level of 170 mm. progresses uniformly with increasing lung distention until the low level of 36 mm. is reached.

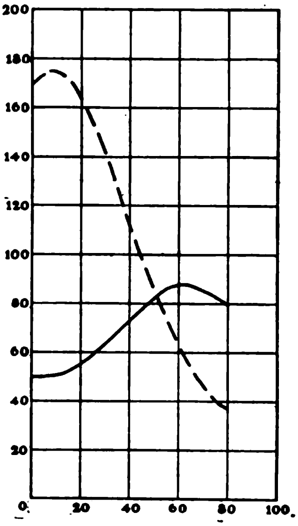

Fig. 3. Plotted curves showing corrected carotid (broken line) and pulmonary (unbroken line) arterial pressures during progressive decrease of intraplethysmographic pressure. Ordinates indicate blood pressures. Abscissae indicate decrease of plethysmographic pressures in millimeters of mercury.

The pulmonary arterial pressure, on the contrary, is not noticeably affected by slight distention of the lungs, and with increasing distention rises continuously until its maximum is attained, which occurs when the increase in the intrapulmonic air pressure is equal to 60 mm. of mercury. Beyond this point the pulmonary pressure commences to fall. The maximum increase in the pulmonary pressure is 40 mm. above the preëxperimental level of 50 mm. and the relatively high level of 90 mm. is attained.

It will be noted that the pulmonary pressure rising from a low level becomes equal to the carotid pressure falling from a high level at a point where the pressure in the two systems is equal to 85 mm. The increase in intrapulmonic air pressure sufficient to effect this equality is 50 mm. of mercury. With increase in air pressure in excess of 50 the pulmonary pressure continuing to rise attains a level 46 mm. above that of the falling carotid pressure.

An interesting observation is that both the fall in carotid and the rise in pulmonary pressures are, within a wide range of lung distention, approximately linear functions of the increasing intrapulmonic air pressure.

DISCUSSION OF RESULTS

While the pulmonary arterial pressure cannot under usual living conditions be taken as an index of the right ventricular diastolic pressure, yet, under the condition of completely blocked pulmonary capillaries the right ventricular pressure must rise *pari passu* with the rise in pressure in the pulmonary arteries and this rise must be sustained in the ventricle even during its diastole. Blood under high pressure is trapped between the blocked capillaries at one end of the system and the tricuspid valve at the other. When the pressure in the pulmonary artery equals the maximum which the right ventricle is capable of establishing, subsequent contractions are unable to discharge blood, and the ventricle also remains constantly full of blood under a pressure equal to that in the pulmonary arteries. Even during diastole no relief from this excessive pressure is obtained since the ventricle cannot, even though relaxed, increase its capacity for holding blood. It is even quite possible that under these conditions the pulmonary semilunar valves fail to close, since the normal tendency of the blood to regurgitate is absent when the ventricle is full of blood at the end of its systole. In evidence of the diminished output of blood from the right side of the heart when the pulmonary capillaries are mechanically blocked by distention of the lungs, a tracing (fig. 1) is shown in which the pulse is obliterated in the record from the pulmonary artery while the mean pulmonary pressure rises. Obliteration of the pulse waves occurs in the carotid as well as in the pulmonary tracing but in one case it is due to insufficient and in the other to excessive pressure.

When the pulmonary capillaries are completely blocked the pulmonary arterial pressure may then be taken as a direct measure of the diastolic right ventricular pressure and it may be assumed that with an incomplete blocking of the capillaries there exists a rise in the diastolic right ventricular pressure corresponding in degree to that maintained in the pulmonary arteries. Two of the tracings shown in figure 2 are evidence of the fact that with moderate distention of the lungs the rise in mean pulmonary arterial pressure is due to a rise in diastolic rather than in systolic pressure. In the third tracing of this series it will be noted that as the pulmonary blood pressure rises the output from the ventricle diminishes. That the diminution in ventricular discharge is not due to a diminished supply of blood to the auricle and ventricle was evidenced by many post-mortem examinations which revealed not only a dilated right ventricle but also an engorgement limited to the right auricle and adjacent veins in those animals that were killed by excessive increase of intrapulmonic pressure. It will be noted in figure 3 that after attaining its maximum the pulmonary arterial pressure commences to fall. This failure is attributable to cardiac failure secondary to impairment of the coronary circulation. Marked fall in carotid pressure necessarily affects the coronary circulation of the entire heart but when the right ventricle is distended during its diastole with blood under high pressure, the impairment of circulation through the cardiac muscles is far greater in the right ventricle than in other parts of the heart.

Taking the pulmonary arterial pressure as an index of safety during distention of the lungs, reference to figure 3 shows that with 50 mm. increase in the pressure of the intrapulmonic air the carotid and pulmonary arterial pressures become equal and that with increasing air pressure the pulmonary pressure becomes higher than that in the carotid artery. The limit of safety, therefore, in dogs lies below this degree of pressure and death has been found not infrequently to follow air pressures of 30 and even less.

There is no reason to believe that the pulmonary capillaries of man are more resistant to occlusion than are those of the dog or that there exists much difference in their relative blood pressures. Difference in the elastic factor of thoracic expansion is not important since increase in the intrapulmonic air pressure acts almost directly on the lung capillaries independently of lung expansion. The observations, therefore, made on dogs require little or no modification to become pertinent to artificial respiration performed on man by any of the methods that increase intrapulmonic air pressure. When the lungs are rhythmically distended an important factor of safety is the shortening of the period of full inflation to allow the blood pressures to return to normal.

CONCLUSIONS

1. When in dogs the lungs are artificially distended by an increase in the intrapulmonic air pressure:

a. The pulmonary arterial pressure becomes equal to that of the general systemic arterial pressure when the excess of intrapulmonic pressure is equal to 50 mm. of mercury.

b. With further increase in intrapulmonic pressure the pulmonary arterial pressure may exceed that of the general system by as much as 46 mm. of mercury.

c. Under both of the above conditions the circulation of blood through the coronary arteries of the right heart is unfavorably influenced.

d. With the rise in pulmonary arterial pressure there occurs a rise in the diastolic right ventricular pressure. When the latter becomes equal to or greater than the systemic diastolic pressure, circulation of blood through the coronary vessels of the right ventricle becomes impossible.

2. Estimates of the increase in intrapulmonic air pressure sufficient to influence unfavorably the coronary circulation of dogs are applicable also to man and should be considered in connection with methods of artificial respiration.

We are indebted to Prof. W. E. Garrey for helpful suggestions and criticisms.

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VAGUS AND SPLANCHNIC INFLUENCE ON THE GASTRIC HUNGER MOVEMENTS OF THE FROG. COMPARATIVE STUDIES III[1]

T. L. PATTERSON

From the Hull Physiological Laboratory, The University of Chicago, and the Physiological Laboratory, Queen's University

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INTRODUCTION

The character of the continuous motor activity of the empty and filled stomach in the frog has been reported (1). In the present paper an attempt is made to determine more specifically the influence of the vagi and splanchnic nerves on the behavior of the gastric hunger movements and the gastric tonus of the empty stomach.

Much has been written concerning the excitatory and inhibitory influences of these extrinsic nerves upon the gastric motility and a rather thorough review of the literature covering this phase of the question has appeared in a previous paper of this series (1). In addition, the distribution and function of the nerves innervating the visceral and vascular systems in crocodiles and alligators was worked out by Gaskell (2). He found that stimulation of the peripheral end of either vagus above or below the ganglion trunci vagi invariably led to a contraction of the stomach musculature. After section of the cervical vagus above the ganglion with subsequent degeneration of its fibers stimulation then above the ganglion almost invariably produced no effect whatever on the esophagus and stomach, while stimulation below the ganglion almost invariably caused marked peristaltic contraction. Gaskell came to the conclusion that the fibers which innervate the thoracic portion of the esophagus and the stomach and all probability the intestines, degenerate only in that portion which is above the ganglion but not in that portion below the ganglion and that the nerves for the upper part of the esophagus and the inhibitory fibers of the heart have no connection with the nerve cells of the ganglion trunci vagi, while the motor nerves for the rest of the esophagus and the upper portion of the remainder of the alimentary canal are in connection with the cells of that ganglion—a connection by which the motor fibers proceeding peripherally from the ganglion are prevented from degeneration but not the motor fibers which pass to the ganglion.

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[1] A preliminary report of this work was made before the 1917 meeting of the American Physiological Society at Minneapolis, a brief abstract of which was published in the Proceedings of that Society.

Furthermore, the recent observations by Crohn and Wilensky (3) on gastric behavior by the balloon method have shown that in atony of the stomach the hunger contractions disappear and in advanced cases the tonal waves also, while in purely secretory or other functional disturbances both kinds of waves persist.

EXPERIMENTAL PROCEDURE

The studies in this series of experiments were made upon the large bullfrog (Rana catesbiana). All the animals were provided with an artificial opening into the posterior part of the mouth or stomostomy and the movements of the empty stomach were recorded by the balloon method as described in a previous paper (4). A series of normal contractions of the empty stomach was obtained from each animal which extended over a period of several days and then each of these animals was operated on a second time. In this second operation, either both vagi or both splanchnic nerves, or both the vagi and the splanchnic nerves together were sectioned, followed after recovery in each case by a series of tracings from the empty stomach. The animals were anesthetized. Aseptic precautions as far as possible were at first followed but later this was found to be unnecessary as no infection developed in any of the animals when such procedure was not followed. The vagi were sectioned in the region of the neck. Two oblique incisions were made through the skin on either side of the median line, ventral, about 1 cm. distant and close to the anterior tips of the shoulders as represented by a line drawn from this point laterally 1 cm. to 1¼ cm. in length to a point slightly posterior and just internal to the articulation of the superior and inferior maxillary bones on either side. These two incisions exposed the cervical fascia on either side at its attachment along the anterior scapulo-clavicular borders. Here there are few blood vessels and if the fascia is carefully separated no hemorrhage results. As soon as this region is passed the fascial separation becomes very easy until the thin sheet of prevertebral fascia is reached which is about on a line of the transverse processes passing obliquely downward and inward from the base of the skull and extending into the thorax. This latter sheet of fascial membrane is now pierced which exposes the levator anguli scapulae muscle, over the anterior border of which courses the vagus nerve and the internal jugular (Vena jugularis) and musculo-cutaneous (Vena musculo-cutanea) veins. The incision is held open by the spring of a small pair of forceps (preferably curved points) and then by means of a small pair of mouse-toothed forceps the nerve is carefully separated from the adjoining veins to which it is bound by connective tissue. This is best accomplished by freeing the nerve either between the two veins mentioned or just lateral to the internal jugular vein at the anterior border of the levator anguli scapulae where it crosses and sectioning the nerve just below the origin of the recurrent laryngeal branch. Section of the nerves at this point destroys not only the gastric branches to the stomach but also the pulmonary and cardiac branches destined for the lungs and heart. Attempts were made at first to section only the gastric branches but as these branches were so small and so deeply embedded in the tissues it was found to be practically impossible with recovery of the animals. In fact, the technic as used required several months' experience before it became perfected and only then did it become an efficient procedure which, if properly handled, may be called a bloodless method. Both vagi were always sectioned at one operation and the skin incisions were closed with five sutures.

The splanchnic nerves were sectioned in the region of the coeliac plexus after laparotomy. An incision was made through the skin, the rectus abdominis muscle and the aponeuroses of the external and internal oblique muscles 2½ to 3 cm. in length extending from the lower extremity of the sternum (xiphisternum) caudalward and about ½ cm. to the left of the linea alba, in order to avoid the anterior abdominal vein (Vena abdominalis) which courses forwards along the mid-line of the ventral body wall until opposite the liver. The stomach is withdrawn through this opening and a larger pair of nerves, one on either side, is found coursing along with the right and left systemic arches. These are the third spinal nerves carrying fibers for the stomach, but according to Steinach and Wiener (5), Dixon (6) and others, the stomach also receives fibers from the fourth and fifth spinal nerves and Waters (7) in addition includes the sixth.

Fibers from these nerves arising from both sides of the body unite to form the coeliac plexus situated on the coeliaco-mesenteric artery

(Ateria intestinalis communis) a few millimeters from its origin from the left systemic arch. From this plexus arise the nerves destined for the stomach, pancreas and duodenum. The branches for the stomach are inbedded in the mesenteric membrane and follow closely the course of the arterial supply of this organ. They may be best seen by raising the stomach and allowing the light to illuminate the mesentery when they may be picked up with mouse-toothed forceps. If they are cut close to the plexus there are usually not more than two branches. Experience has shown that it is advisable to introduce into the stomach 5 or 6 cc. of water previous to the operation as it tends to fill and round out the stomach, thus making it easier to locate the plexus and the nerves. The technic for this operation like that for double vagotomy in the frog is very delicate, but with sufficient patience and experience it may be developed to such a point as to be conducted without hemorrhage, and like the former may be considered a bloodless method. The splanchnetomized stomach is pushed back into place, the muscular incision is closed with nine to ten sutures and the skin incision with the same number. The animals after double vagotomy or splanchnetomy are usually sufficiently recovered on the third day following the operation to be used for experimental tests with fairly marked gastric activity, while after a double operation consisting of the two above they are usually not ready for use until the fourth day following the operation. In the decerebration experiments the balloon was not removed from the stomach and the gastric contractions started again after a short period of inhibition. All the tracings were recorded on a slowly moving drum making a revolution in fifty to sixty minutes.

THE INFLUENCE ON THE GASTRIC HUNGER MOVEMENTS OF PARTIAL AND COMPLETE ISOLATION OF THE STOMACH FROM THE CENTRAL NERVOUS SYSTEM

A complete knowledge of the mechanism of the gastric movements is still uncertain. The gastric activity is regulated not only by the vagi and the splanchnic nerves of the sympathetic system, but also by the automatically acting plexi of Auerbach and Meissner. The most direct and desirable method of attack on this problem is the section of the extrinsic nerves to the stomach, although this operation abolishes not only all direct influences from the brain of a motor or inhibitory type, but also the central reflexes (motor or inhibitory) that may be called into action through the sensory nerves in the stomach.

The influence of these nerves on the activity of the stomach has been studied by a number of investigators, prominent among whom have been Cannon (8) and Carlson (9). Cannon's observations on the gastric movements of digestion in cats have shown that section of the vagi leads to a temporary loss of tonus and a slowing and weakening of the peristalsis, which in respect to rate is practically restored in a few days. He infers as does Kelling (10) that their function is solely to make the gastric muscles exert a tension (tonic state) and the result of this condition is peristalsis. Furthermore, section of the splanchnic nerves does not affect the movements of digestion, while the combined vagi and splanchnic section leaves the digestive movements of the stomach practically normal even shortly after the operation.

Carlson, on the other hand, has shown that section of the vagi in dogs leaves the empty stomach on the whole permanently hypotonic, at least for a period up to three months after the operation. Section of the splanchnic nerves increases the gastric tonus and augments the gastric hunger contractions, while the section of both the vagi and the splanchnics leads to a permanent hypotonus of the stomach, except under conditions of prolonged fasting. These discrepancies between the results of the two investigators are probably accounted for, in that the tonus of the vagus plays a greater rôle in the movements of the empty than in the movements of the filled stomach, or else the nerves vary in different species of animals.

1. The effect of complete section of the splanchnic nerves. Complete section of the splanchnic nerves on both sides in the region of the coeliac plexus was made on twelve frogs and after recovery from the operation records of the movements of the empty stomach were continued from two to three weeks and compared with those from the normal stomach of the same animal.

When a comparative study of the records of these animals is made as a whole it is evident that the complete section of the splanchnic nerves with the vagi intact in frogs increases markedly the gastric tonus and augments the movements of the empty stomach (fig. 1, *A* and *B*). The recorded contractions are small, rapid and irregular in form, and represent virtually an incomplete or hunger tetanus of the stomach. In other words, the stomach on the whole becomes strongly hypertonic and more active through the destruction of the inhibitory fibers via splanchnic nerves to the stomach, which permits the motor fibers of the vagi to exert their full influence on the gastric motor mechanism, thus leading to a high degree of gastric tonus much above the normal.

This particular state of excessive tonus is evidenced not only by the balloon in the stomach cavity but more especially by the marked contraction of the esophagus and stomach as is exhibited many times by the extreme difficulty to introduce the balloon through the esophagus into the stomach. This hypertonic condition of the frog's stomach which always appears after complete section of the splanchnic nerves is evidently more marked than Carlson (9) found it to be in dogs after splanchnic section. Furthermore, this condition as it exists in frogs after this type of nerve section corresponds apparently to certain clinical conditions as reported by Eppinger and Hess (11) under the term of vagotonia. According to these observers the antagonistic influences between the gastric branches of the vagi and the spanchnic nerves play a very important rôle in not only moderating the physiological impulses which might reach a very marked intensity, but in addition they prevent acute transitions from rest to excitation or vice versa. This means that if it were not for the above under certain conditions small stimuli might cause large reactions either physiological or pathological. Now,

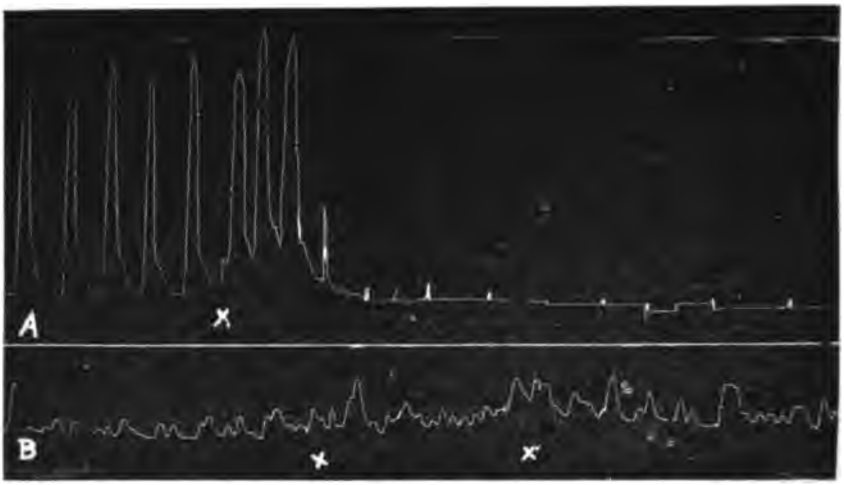

Fig. 1. Records from the empty stomach of the frog. *A*, normal frog after six days' fast; *B*, the same animal ten days after section of both splanchnic nerves and twenty days' fast. At *x*, introduction of 5 cc. of 0.5 per cent solution of hydrochloric acid directly into the stomach. Showing incomplete tetanus and only slight inhibition of the hunger contractions by acid in the stomach after section of the splanchnic nerves. *x'* = termination of the acid injection.

if we assume that somewhere in the central nervous system there exists a common center which controls the antagonistic actions of these two systems, as suggested by these investigators, and that the irritability of this center increases and decreases from time to time it is easy to understand how very weak and even transitory stimuli might act upon such a center when in a state of increased irritability to produce the gastric hypertonus through the fibers of the vagi. This however is not proven, but we do know that in the condition of vagotonia there is a functional increase of tone via vagi to the stomach and this increase of function doubtless permits the stimuli to act more readily than if the reversed condition existed. Furthermore, the observations of Crohn and Wilensky (3) have shown that the hunger contractions in well-marked cases of vagotonia exhibit an extreme degree of variability, the contractions following one another in rapid succession and without pause for comparatively long periods of time. The clinical findings of these observers are apparently in accord with the results on frogs after splanchnic section.

The inhibition of the movements of the empty stomach of the splanchnetomized animal when acid is introduced into the stomach cavity is much less complete than in frogs with all the extrinsic gastric nerves intact (fig. 1, *A* and *B*). In fact, the contractions do not cease at all and the only effect produced is a very slight decrease in the height of the contractions during the introduction of the acid followed by a few contractions of a slightly longer duration and usually a slight increase in the gastric tonus. This diminution of the inhibition following stimulation of the gastric mucosa by acids after complete section of the splanchnic nerves is confirmatory with the findings of Carlson (12) on dogs.

2. The effect of section of both vagi nerves. Section of both vago-sympathetic nerves in the neck was made on eleven frogs and after recovery from the operation records of the movements of the empty stomach were continued from two to three weeks and compared with those from the normal stomach of the same animal.

When all the records are compared from these animals the results are confirmatory in showing that the contractions of the empty stomach are only slightly changed in rate and regularity. The contractions when viewed as a whole resemble those from the normal stomach with the exception that they usually appear to be of a slightly slower rate, weaker and more irregular (fig. 2, *A* and *B*). However, there is a tendency for the contractions to increase in strength or rather amplitude up to the amplitude of the normal contractions and some of the individual contractions may even exceed the normal. This is evidently produced through a lowered tone in the gastric motor mechanism, whereby the contractions start rather suddenly and without any marked preliminary increase in tonus and because of this condition the air is more completely forced out of the balloon, thus resulting in the greater contraction. In the empty stomach of the normal animal as determined by the balloon method, there are practically no tonus changes, or at least they are so slight in degree as to be almost a negligible factor. In working with these animals, extending over a period of five years, I have never observed in the normal animal an increase in gastric tone exceeding a cen-

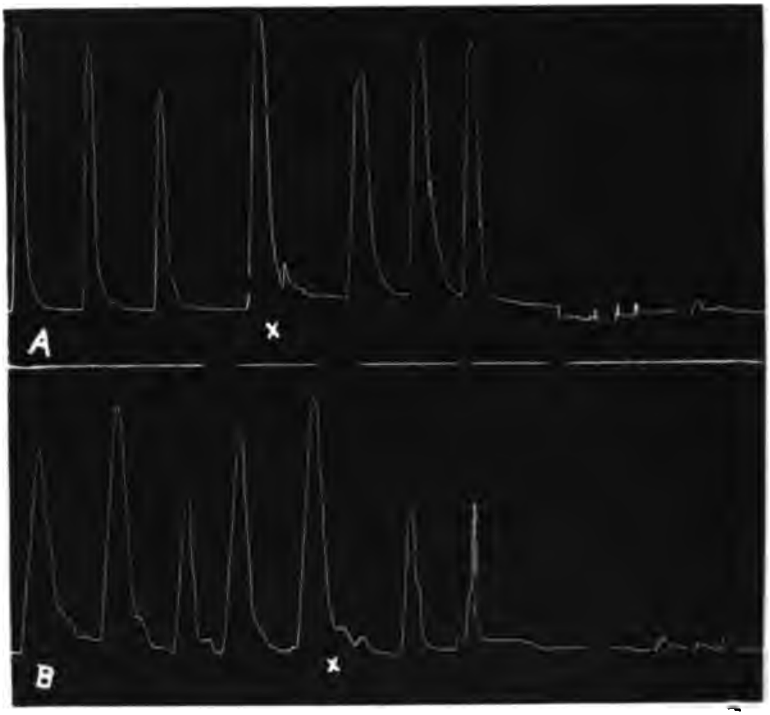

Fig. 2. Records from the empty stomach of the frog. *A*, normal frog after three days' fast; *B*, the same animal nine days after section of both vagi and sixteen days' fast. At *x* introduction of 5 cc. of 0.5 per cent solution of hydrochloric acid directly into the stomach. Showing slightly more complete inhibition of the hunger contractions by acid in the stomach after section of the vagi nerves.

timeter as determined by the manometric pressure. This maximal increase I have observed not more than three or four times in my work during this time and is therefore rare. When tonus changes are observed they usually do not exceed a quarter of a centimeter (2 to 3 mm.), but the more common thing in the frog is to have the tonus remain constant hour after hour.

Section of both vagi with the splanchnics intact leads to a sympatheticotonic condition of the stomach. This means that the stomach on the whole becomes hypotonic through the destruction of the vagal fibers that maintain the gastric tonus, which permits the inhibitory fibers of the splanchnics to exert a greater influence on the gastric motor mechanism, thus leading to a general diminution in the gastric tonus. However, there was a tendency in some of the animals, at least, to show a gradual improvement in the efficiency of the local tonus mechanism as time went on after the operation, which indicates that the hypotonic condition of the stomach may be only temporary in the frog and not permanent as reported by Carlson (9) in dogs, but corresponding to the observations of Cannon (8) in cats for the movements of digestion. This phase of the question will be discussed in a separate paper. Furthermore, the gastric tonus on the whole is much lower than normal as determined not only by the balloon in the gastric cavity, but also by the ease with which the balloon may be introduced through the esophagus into the stomach and inflated.

When acids are introduced directly into the empty stomach of the vagotomized animal gastric inhibition is exhibited similar to that produced in the normal animal with the exception, on the whole, that it appears to be quicker and more marked than in the normal animal (fig. 2, *A* and *B*). This is exactly contradictory to Carlson's results on dogs (9), yet he states that this was what he expected to find, namely—an augmentation of the inhibition through the splanchnics after section of the vagi.

3. The effect of complete section of the vagi and splanchnic nerves. Combined splanchnic and vagi sections were made on ten frogs and after recovery from the operation records of the gastric movements were continued from two to three weeks and compared with those from the normal stomach of the same animal. Both sets of nerves were sectioned at the same operation.

After this complete isolation of the frog's stomach from the central nervous system the movements of the empty stomach are much the same as when the vagi alone are severed. The contractions show a tendency to approach or even in some cases to exceed the normal, while at times they may even be identical in rate and character with those of the intact stomach, but on the whole they are of a slightly slower rate and more irregular. The stomach passes into a hypotonic condition similar to that after section of the vagi, and therefore the slight changes in the movements of the empty stomach after isolation from the central nervous system must be due primarily to the persistent hypotonus. These results are in general confirmatory with those of Cannon (8) on cats and Carlson (9) on dogs.

The inhibition of the movements of the empty stomach by acid stimulation of the gastric mucosa persists after complete isolation of the stomach from the central nervous system, but the inhibition like that found by Carlson (12) in dogs is diminished in intensity and duration. There is a gradual and slow diminution, both in the rate and amplitude of the hunger contractions but as a rule this does not produce complete inhibition in the frog. The inhibition is therefore primarily a local reflex determined by the local gastric mechanism rather than by the character of the central innervation or the central inhibition. Since the type of gastric activity after complete isolation of the stomach from the central nervous system does exhibit the typical movements of the empty stomach, the primary stimulus to these contractions is not to be sought in the extrinsic nerves. The extrinsic nerves (vagi and splanchnics) must therefore be considered under normal conditions to play the important rôle of modifying or regulating a primary automatic mechanism in the stomach wall.

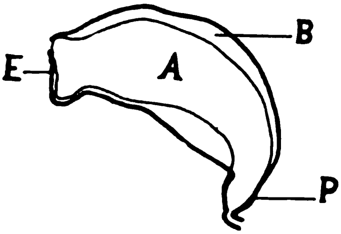

Fig. 3. Splanchnetomized stomach superimposed upon the vagotomized stomach from two frogs of equal size, weight and vigor. *A*, splanchnetomized stomach. *B*, vagotomized stomach. *E*, esophagus. *P*, pyloric portion of stomach. Note the hypertonic condition of stomach *A*.

4. Extirpation of stomachs after section of vagi and splanchnic nerves. Early in the course of the investigation it was observed that the stomachs of splanchnetomized and vagotomized animals exhibited rather wide variations in size, depending on whether the vagi or the splanchnic nerves had been previously severed. This phase of the problem was investigated on ten splanchnetomized and ten vagotomized animals and the stomachs were removed from three to ten days after the operation, directly after the killing of the animals and while the hearts were still beating. In the selection of these animals care was taken to select frogs of equal size, weight and vigor. The results of these experiments are conclusive in showing that the same general influence which the vagi and splanchnic nerves exert separately on the gastric apparatus may be shown when the splanchnetomized stomach is superimposed upon the vagotomized stomach from two frogs of equal size. The latter or larger stomach represents the atonic and the former or smaller the hypertonic, while the normal stomach takes an intermediate position between the two (fig. 3). It may be said, therefore, that the reciprocal or contrary innervation of Meltzer which may be termed antagonistic tonus, may be physiological as long as it serves the purposes of the organ in question in a beneficial manner. It is pathological as soon as the tonus of one or the other is so exaggerated that the common welfare of the organ is in danger, and that is exactly what happens in the splanchnetomized frog's stomach where the hypertonus of the vagus leads to a state of over-excitability, or to the Eppinger-Hess condition of vagotonia.

PSYCHIC OR REFLEX INHIBITION OF THE GASTRIC HUNGER MOVEMENTS

It was suggested to me by Doctor Rogers early in the course of this investigation that it might be well to study certain cerebral processes in relation to the reflex effects on gastric activity. Previous work on other animals has demonstrated that anything which interests, annoys, frightens or angers, leads to a temporary inhibition of the gastric hunger contractions probably via splanchnics. Furthermore, the sight or smell of food in the dog, at least, leads to this same temporary inhibition if not too often repeated. In order to test further the very important reflex control of the gastric hunger mechanism, as well as of the nervous foci in the medulla, mid-brain and cerebrum concerned in the conduction of sensory and motor hunger impulses, the effects of sound and light stimuli were made use of in the following experiments. The observations were made on six frogs which were later decerebrated and the observations repeated. In the case of the sound stimuli, whistles of different pitches were sounded for periods of from ten to twenty seconds but these caused only very slight gastric inhibition which was of short duration and after two or three repetitions it invariably became ineffective, thus defeating the object of the experiment. Even the filing of a glass rod on the table containing the animal was fully as ineffective in producing inhibition although a second factor must have been introduced, that of vibration. All of these stimuli were of minimal or very moderate intensity and evidently not of sufficient strength to produce an effective and constant reflex, or else the central nervous mechanism for this reflex is at a low degree of development.

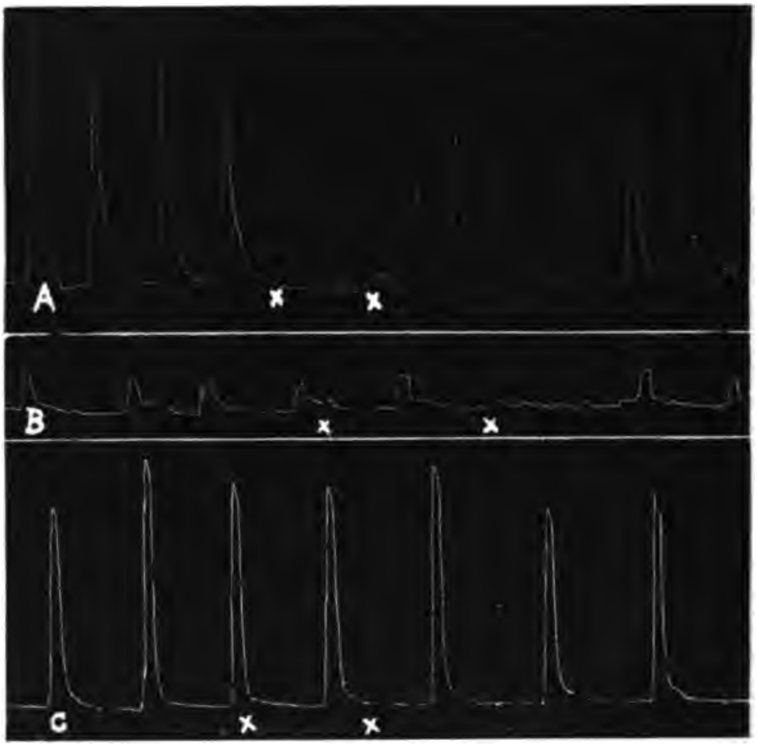

Fig. 4. Records from the empty stomach of frogs. *A*, frog nine days after section of both vagi with splanchnics intact; *B*, frog eight days after section of both splanchnic nerves with vagi intact; *C*, frog nine days after section of the vagi and splanchnic nerves. *x* to *x*, light and darkness shadow test showing temporary inhibition in *A*; very slight inhibition in *B*; and total absence of psychic or reflex inhibition in *C*, in case of the stomach isolated from the central nervous system.

The light stimulus, on the other hand, proved to be more effective. The room was darkened by drawing the shades and while the normal hunger contractions were being recorded the animal was carefully uncovered and the bottom of the window shade directly opposite drawn back and forth quietly and at a moderate rate, thus casting light and dark shadows upon the animal. This invariably produced temporary inhibition of the movements of the empty stomach and there seemed to be no diminution in the degree of the inhibition after repeated trials. After removal of the cerebral hemispheres there was total absence of the reflex inhibition and the contractions went on uninterrupted. It would appear that the central nervous mechanism for this reflex was more highly developed than that for sound. In other words, from the animal's standpoint it may be considered an important defensive reflex to warn it of its avian enemies as they soar through the air, thus anxiety and fear leading to the characteristic temporary inhibition of the gastric hunger movements.

When the light and darkness shadow test was applied to an animal after complete section of the vagi with the splanchnics intact it invariably led to a temporary inhibition of the gastric hunger movements via splanchnic nerves (fig. 4, *A*). If the same test was applied to an animal after complete section of the splanchnic nerves with the vagi intact it invariably led to only a very slight inhibition of the gastric hunger movements, as represented by a slight and transitory weakening of the contractions (fig. 4, *B*). This slight degree of inhibition usually in evidence after section of the splanchnic nerves is probably due to the action of the few inhibitory fibers in the intact vagi or to some central inhibition of vagus tonus. In the case of the stomach completely isolated from the central nervous system (vagi and splanchnic nerves cut) there is total absence of any psychic or reflex inhibition since the efferent nerve pathways to the stomach have been broken by the sectioning of all the extrinsic nerves (fig. 4, *C*).

CONCLUSIONS

1. Complete isolation of the frog's stomach from the central nervous system leads to hypotonus of the stomach with about the normal type of gastric hunger contractions. This is in confirmation with the work of Carlson on dogs. The automaticity of the gastric mechanism is independent of the extrinsic nerves but these nerves play an important rôle in modifying or regulating the automatic mechanism in the stomach wall.

2. Partial isolation of the stomach from the central nervous system interrupts the normal antagonistic balance between the vagi and splanchnic systems which may lead to pathological reactions such as vagotonia

after complete section of the splanchnic nerves. The stomach in this condition becomes strongly hypertonic while after complete section of the vagi with the splanchnics intact the stomach passes into a hypotonic condition.

3. The acid inhibition of the movements of the empty stomach by stimulation of the gastric mucosa persists after complete isolation of the stomach from the central nervous system, but the inhibition is diminished in intensity and duration. When the splanchnics alone are sectioned the inhibition is even less marked, but after section of the vagi with the splanchnics intact there is, on the whole, a slight augmentation in the inhibition via these nerves. This latter statement is contradictory to the findings of Carlson on dogs, while the other facts above are in accord.

4. The light and darkness shadow test invariably produces psychic or reflex inhibition of the gastric hunger movements in the normal animal. After decerebration or after complete isolation of the stomach from the central nervous system there is total absence of the light reflex on the gastric mechanism. When the vagi alone are sectioned temporary inhibition is the result, while after section of the splanchnics with the vagi intact there is only very slight inhibition produced.

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OBSERVATIONS ON THE RELATION BETWEEN EMOTIONAL AND METABOLIC STABILITY

FREDERICK S. HAMMETT

From the Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania

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The studies of Cannon (1) and his co-workers on the effects of the major emotions on the physiological activities of the vegetative nervous system have established and clarified certain fundamental phenomena of general biological importance that are wide-reaching in their application.

While the primary consequences of emotional stimulation have been extensively studied, the secondary involvements such as might be evidenced in the variability of the intermediary metabolism have yet to be studied. The emotional ignition of the vegetative neural complexes can hardly be supposed to be limited to a circumscribed reaction but must indeed reverberate throughout the organism as a whole.

It is obvious that it is possible to divide mankind into two main groups according to temperament or relative emotional stability, between the extremes of which there may exist all gradations of susceptibility to emotional excitation. There are those who are relatively emotionally stable, who pursue the even tenor of their way apparently and actually undisturbed by the surrounding daily happenings. And there are those whose temperaments are of the hair-trigger type, whose emotions are always on tap and who respond to the slightest stimulus with a magnitude of reaction all out of proportion to the value of the stimulus received. Whatever the causes of these differences of susceptibility and response as evidenced in the differences of emotional stability, the results on the recipient and effector organism must of necessity be widely different. That these responses primarily involve the vegetative nervous system has been definitely demonstrated. That they secondarily involve the intermediary metabolism should be expected since the processes of digestion, secretion, absorption, utilization and excretion are all directly or indirectly bound up with the directing or controlling influence of this part of the nervous system.

During the past year opportunity was afforded the writer to study the chemical composition of the blood of emotionally stable and unstable insane and normal persons. The classification of the emotional status of the individuals presented in this study was arrived at by consultation with the physicians in actual contact with the patients and by personal observation over considerable periods of time. The analyses of the bloods were carried out as reported in a previous communication (2) and for the series under discussion here were made at weekly intervals on the seventeen subjects reported. The duration of the periods of observation varied from three to six weeks.

TABLE 1

The coefficient of variability of the blood constituents and the total metabolic variability of the subjects studied

	TOTAL N	NON-PROTEIN N	UREA N	CREATININE N	CREATINE N	URIC ACID N	AMINO ACID N	REST N	SUGAR	TOTAL VARIABILITY
1	7.21	12.81	14.15	5.68	12.54	14.13	7.72	47.78	7.85	129.87
2	5.07	8.09	11.23	6.56	11.15	16.07	32.23	19.19	15.51	125.10
3	11.40	6.84	13.11	8.66	21.60	7.77	16.33	24.07	12.21	121.99
4	11.32	10.73	22.30	5.89	6.56	15.77	16.17	18.02	6.72	113.48
5	11.70	8.19	8.67	3.93	6.96	26.70	4.21	28.13	7.89	106.38
6	8.35	7.71	14.10	4.81	14.82	13.69	10.80	23.42	8.63	106.33
7	10.65	3.99	14.67	4.97	17.50	6.43	6.27	25.54	16.00	106.02
8	2.18	12.86	12.57	7.06	7.00	4.94	11.71	23.48	16.91	98.71
9	9.64	11.33	18.90	6.57	6.26	13.47	12.73	12.16	7.48	98.54
10	3.63	6.22	9.52	5.83	7.32	11.59	17.97	19.19	15.87	97.14
11	9.38	8.87	4.69	1.33	5.54	13.38	5.77	39.72	3.98	92.66
12	1.08	2.19	8.25	4.31	5.90	7.81	3.84	45.03	5.75	84.16
13	3.56	8.78	18.05	4.81	3.64	9.33	3.37	19.47	12.86	83.87
14	10.10	5.22	11.98	3.71	8.80	3.95	17.32	6.06	13.16	80.30
15	9.18	6.96	11.76	6.52	7.28	6.02	10.96	7.50	12.72	78.90
16	8.73	4.18	1.43	12.61	5.74	11.00	10.16	10.84	13.02	77.71
17	17.35	4.98	5.95	4.09	10.06	2.19	8.85	13.58	9.32	76.37

As a basis of correlation between the emotional and metabolic stability, the coefficient of variability (3) for each blood constituent determined for each individual was calculated and the sum of these coefficients was taken as the total variability of the intermediary metabolism of the person in question. Table 1 gives these figures for each constituent determined in each subject. The subjects are arranged in the order of their decreasing variability.

An inspection of the table shows that while the differences in variability of one individual from the next is small, there is a very evident marked difference between those of highest and of lowest metabolic instability, and this marked difference is correlated with a marked difference in emotional reactivity if we consider the subjects seriatim from the psychological point of view.

Number 1 is a male nurse of the small nervous type, easily upset by minor occurrences and with a continual attitude of worry. Number 2 is a female patient, who although being completely oriented, varies in her emotionalism from deep depression with decreased psycho-motor activity to a wild hilarity and excitement. Number 3 is a male patient, restless, talkative, active and excitable, showing much exhilaration and flightiness. Number 4 is a male patient classed as an agitated depressive and who has firmly fixed somatic and autopsychic delusions. Number 5 is a male nurse, irritable, suspicious and touchy, possessing neither decision nor attention. Number 6, the last of those showing a total variability of over 100, is also a male patient, restless, irritable and excitable, showing considerable emotional elation. The next two members of the series are also of the emotionally unstable type, number 8 being a female patient showing considerable perturbation and violence accompanied by motor activity, screaming, laughing and hallucinations, while number 9 is a male presenting a history of hypersensitiveness, and who becomes apprehensive under examination, is easily depressed and worries, although at other times he is more cheerful. All these individuals so far described, then, can be validly considered as persons of varying emotionalism and of obvious emotional instability. They are also individuals whose metabolic variability is of a relatively high grade, as can be seen from the table. Turning now to the remainder of the subjects the next on the list, no. 10, shows a metabolic variability that is practically the same as found in the latter members of the preceding group. Yet his emotional status to all appearances is one of relative stability as far as can be determined. He is pleasant and agreeable and inclined to be seclusive. He is quiet and not irritable. An inspection of the figures obtained for the metabolic stability from now on shows not only gradually decreasing values but also values definitely and markedly lower, as a group, from those preceding. Number 11, a male patient, is quiet and sits as though in deep thought. He is of rather an even temperament, occasionally becomes angry, but is usually able to control himself. Number 12 is a male patient, quiet, maintaining a given posture for some length of time, sits rigidly in a chair and stares vacantly into space. He is indifferent toward his surroundings. Number 13 is a female patient who is emotionally apathetic and indifferent. Number 14, a male patient, is at times excitable but is only apparently superficially disturbed since he eats regularly and well. Number 15, a male patient, was in a catatonic stupor throughout the period of observation and obviously was not emotionally variable, to any determinable extent. Number 16, another male patient, is never excited but is always indifferent, apathetic and seclusive. Number 17, the last of the series, is a male nurse, the emotional antithesis of number 1. He is phlegmatic and inexcitable, paying no attention to the ordinary little vicissitudes of life.

From the psychological point of view it is evident that these latter individuals present the appearance of being relatively emotionally stable. As a group they are generally inexcitable and are not roused to demonstrable emotional reactions by circumstances which act as stimuli causing the marked response of the first group of high metabolic variability.

This relation between a relatively high metabolic stability and a low grade of emotional reaction, and between a relatively low metabolic stability and a condition of temperamental excitability is by no means claimed to be exact or quantitative. Nevertheless the data seem to indicate such a tendency.

The logical conclusion to be drawn from this comparison is that larger variations in intermediary metabolism are prone to accompany conditions of ready emotional response of a marked nature to disturbing stimulation, and that on the other hand the variability of the intermediary metabolism in individuals who are less susceptible is liable to be relatively low.

A broader application of the tendency here demonstrated can be made if for a moment one compares the physical condition of the so-called emotional type of individual with his more phlegmatic and less responsive emotional opposite. The former usually presents a picture of deficient nutrition, the latter is in most cases well supplied with the anabolic products of metabolism. The metabolism of the one by its wide variability gives indications of the possibility of there being at one time an overtaxing of the organism, and at another time of the organism lacking a sufficient energy supply. The other type is relatively more metabolically uniform. His metabolism consists of a balanced give and take, in which no undue strain is put on the catabolic processes nor is there a lack of sufficient material to supply the anabolic needs. The results of these processes are shown in the end products, as indicated by the figures for variability here presented, and the causes are fairly attributable, other factors being absent, to the relative magnitude and type of emotional response of the individual to the incident stimuli.

Such a conception while lacking complete demonstration is nevertheless supported by correlated observations and fits in not only with the observations recorded here but also with the general hypotheses of Cannon (1) as to the susceptibility of the organism to respond to the effects of emotional stimuli by disturbances of the vegetative nervous system.

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FOUR FACTORS CAUSING CHANGES IN THE TYPE OF RESPONSE OF THE ISOLATED INTESTINAL SEGMENT OF THE ALBINO RAT (MUS NORVEGICUS ALBINUS) TO SODIUM CARBONATE

S. HATAI AND F. S. HAMMETT

From the Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania

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The usual response of the isolated duodenal segment from the albino rat intestine is that of shortening or contraction when stimulated by the application of weak solutions of sodium carbonate. Occasionally however segments are encountered that exhibit irregularities in the type of response. One may fail to react to the stimulating substance, another may answer by a preliminary slight contraction which is followed by a relaxation below the original tone level from where the response was elicited, while a third may undergo a prompt and decided relaxation. This last is the irregularity most frequently encountered.

This occasional inconstancy in reaction of segments from apparently normal animals which showed itself at times as a complete reversal of the type of response demanded investigation because it was necessary for our purposes to have rats on hand the intestinal segments of which could be relied upon to give uniform and consistent reactions to stimulation by weak sodium carbonate solutions.

The inconstant responses could not be attributed to variations in the technique of preparation of the segments nor to the carrying out of the tests since this was always uniform. The control of the rats up to the time of killing with respect to age, sex, health, heredity and diet was also in the hands of the authors and consequently these factors were regulated. The preliminary observations which gave satisfactory results had been made on rats which had been for some time in the laboratory cages. Irregularities first began to appear when animals were used before they had become accustomed to the laboratory cages.

Donaldson (1) having reported that the domesticated albino rat is extremely sensitive to changes in environment, we began to think that possibly the change from the colony house to the laboratory had induced an excitement causing the variations. This idea that excitement was the cause of the irregularities was considerably fortified when the segment taken from a rat which had resisted capture and had become quite agitated gave a marked relaxation on stimulation by carbonate. The test of this hypothesis was easy.

The method of procedure for the preparation of the segments for testing was as follows. The rat was first put under light ether anesthesia and then killed by crushing the cord in the cervical region. The duodenal portion of the intestinal tract was then removed without stretching, and cleaned of mesenteric fat. A segment about 1.5 cm. in length was cut from the gastric end of the duodenum and suspended by silk threads in a glass cell containing 4 cc. of Tyrode's solution kept at body temperature and through which oxygen was continually passing. One end of the segment was attached by a thread to a support within the cell and the other end was connected with a light lever writing on a slowly moving drum. Rhythmical contractions immediately appeared and were recorded for one revolution of the drum (about 10 minutes) by which time the tone level had become uniform and consistently parallel with the base line.

With segments prepared in this way the addition of 0.25 or 0.50 cc. of an M/10 solution of sodium carbonate to the Tyrode's solution in the cell usually caused a shortening of the segment with a consequent rise of the curve of rhythmical contractions as is shown in the tracings. It was possible to obtain this type of reaction from one and the same segment for several successive applications of the carbonate. After each application the cell and segment were thoroughly washed with oxygenated Tyrode's solution kept at body temperature. After washing the segment as a rule came back to the original tone level within one revolution of the drum and was then ready for another test. The tests as presented in figures 1 to 5 were obtained in this manner.

In order to determine whether the excitement of the rat brought about either by change of environment or by other means was a factor in causing the irregularities previously described, a number of male rats of the same age and on the same diet were brought to the laboratory cages and allowed to become accustomed to the new environment during three or four days. When a segment from one of the lot was prepared as usual and tracings of the effect of the sodium carbonate stimulation recorded the normal reaction was invariably obtained. When others of the same lot were annoyed by various methods just before killing and similar tracings made the irregularity of reaction occurred

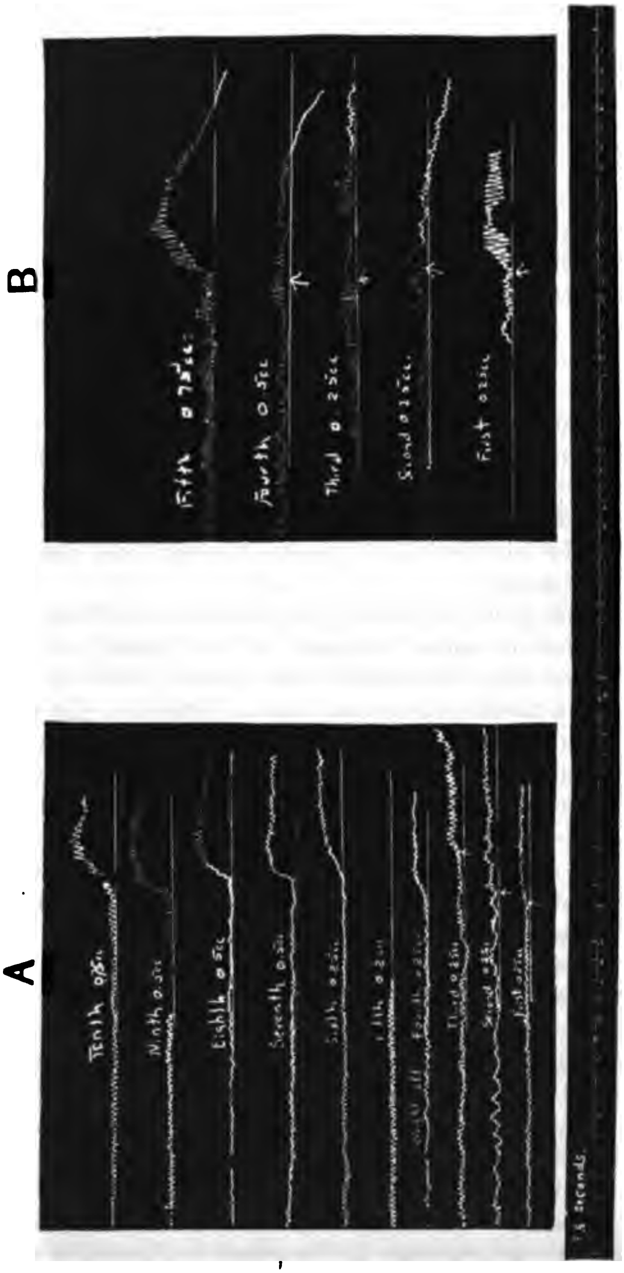

Fig. 1. *A.* Undisturbed normal male rat. Age 100 days. *B.* Disturbed normal male rat. Age 100 days.

in practically every case and usually took the form of a reversal of the type of response as shown in figure 1. Occasionally however an animal was found which did not appear to be susceptible to the preliminary excitation. Such an one is shown in figure 2, rat *C*. Nevertheless the relative frequency of the irregularity was such as to establish the hypothesis that excitement is one of the factors causing variation in the response of the isolated intestinal segment to sodium carbonate stimulation. In this connection it should be noted that although relaxation occurs in the segments from excited animals, this type of response gives way to the normal type after several applications of the sodium carbonate.

The results described in the preceding paragraphs were obtained from rats close to one hundred days old. Desirous of not limiting our material to animals of this age, we attempted to continue the observations on rats one hundred and fifty days old. Here it was found that while evidences of irregularities were present in rhythm, amplitude and base line level, the type of response was usually a normal contraction. When we used a series of rats some two hundred days old it was found that these older animals were quite insusceptible to the effects of the preliminary excitation as indicated by the response of the duodenal segment to carbonate stimulation. This is plainly shown in figure 3. There were no exceptions. It is accordingly evident that the disturbing effect of excitement is modified by age and that immaturity is one of the factors causing susceptibility to excitement with resulting irregularities of response.

The exclusive use of male rats has certain obvious disadvantages. With this point in mind several series of tests were made using as controls undisturbed males and comparing with them females of like age and conditions of environment and diet. It was found that when female rats were used the response of the segment to the sodium carbonate stimulation was normal if the animals were not menstruating. When menstruating, however, as evidenced by congestion of the uterus, variability of response was uniformly obtained and of the same type as that given by segments from the young disturbed male rats. This is shown in figure 4. Hence menstruation is a factor preventing the uniform response to carbonate stimulation. and the test demonstrates that female rats during sexual activity are not suitable material for general use.

The observations up to this point contribute further support to Cannon's (2) theory of the influence of emotional reactions on the vegetative

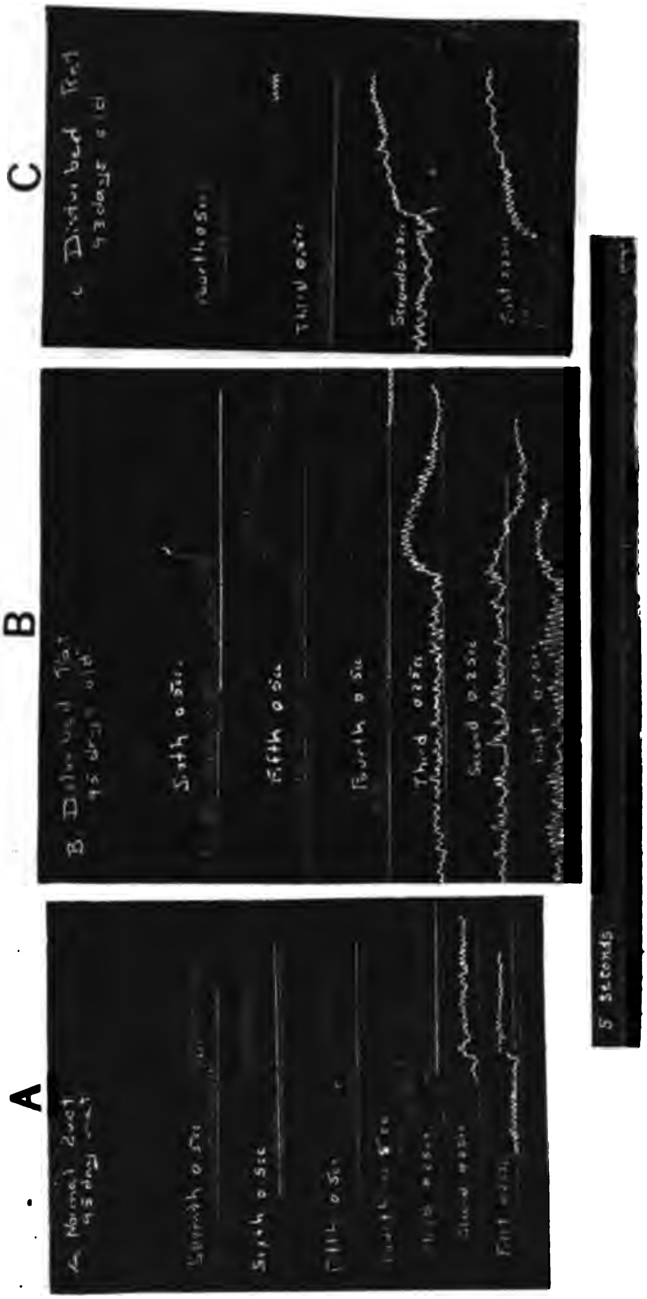

Fig. 2. *A*. Undisturbed normal male rat. Age 95 days. *B*. Disturbed normal male rat. Age 95 days. *C*. Disturbed normal male rat. Age 93 days.

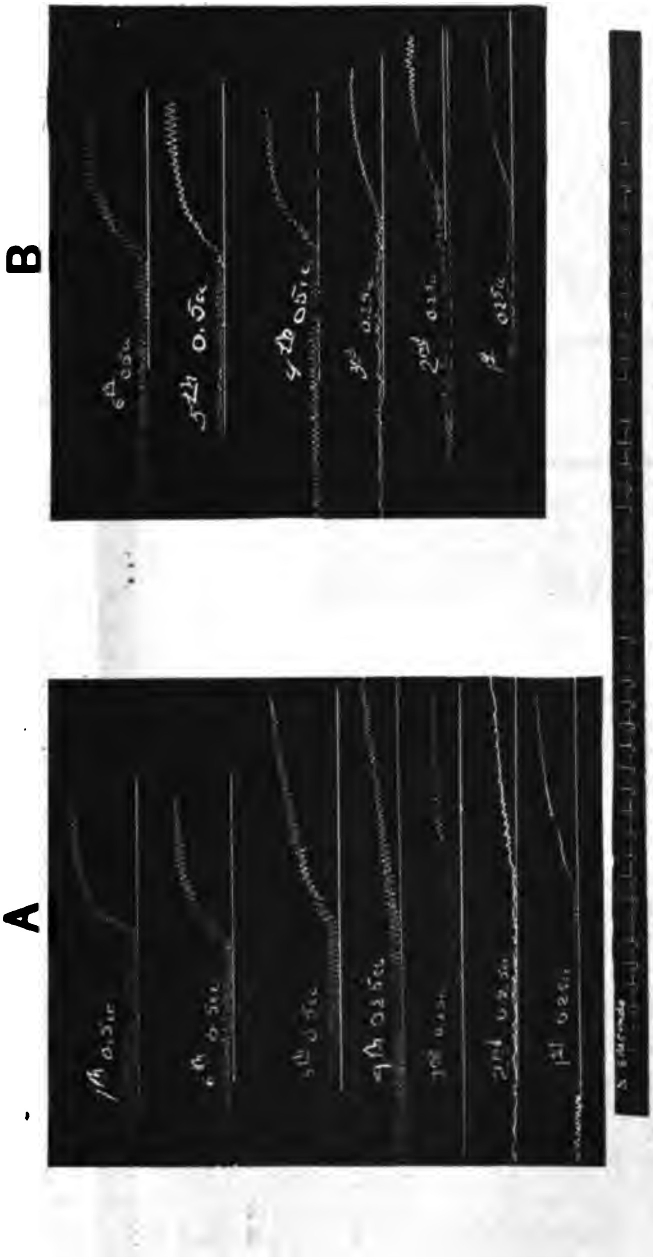

Fig. 3. *A*. Undisturbed normal male rat. Age 200 days. *B*. Disturbed normal male rat. Age 200 days.

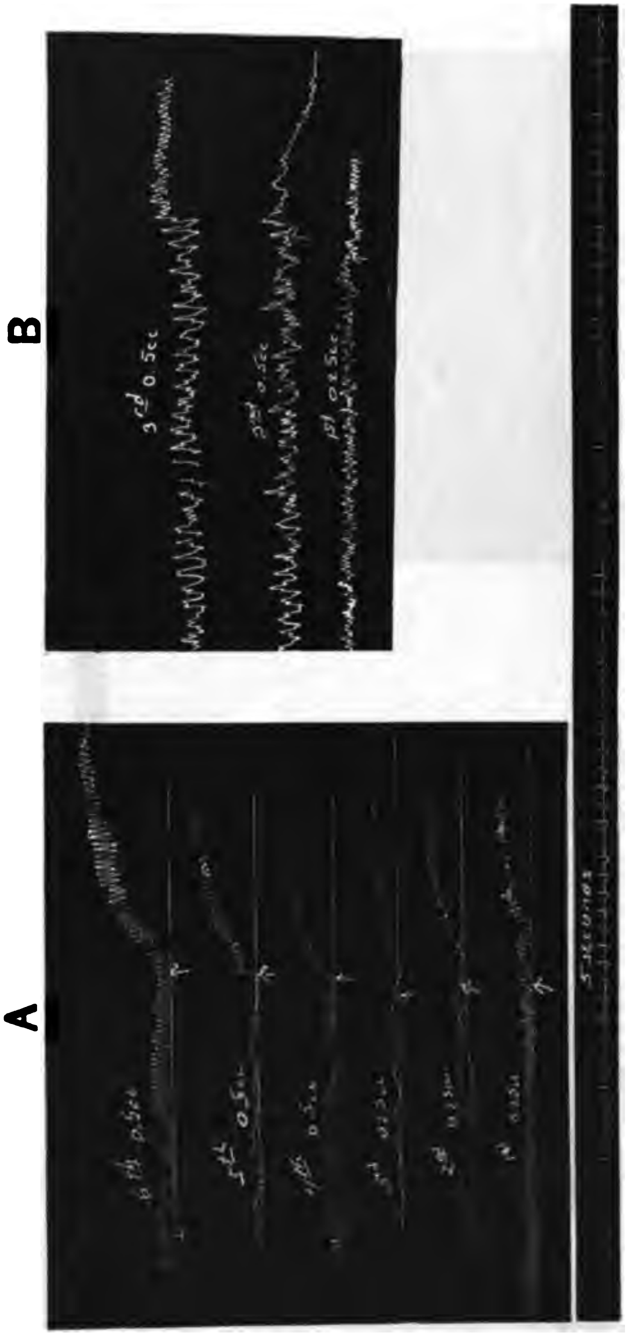

Fig. 4. *A*. Undisturbed normal male rat. Age 200 days. *B*. Undisturbed normal female rat in heat. Age 75 days.

system. They also bear out with more exactness the observation made by Alvarez (3) that the intestine of younger animals (rabbits in his experiments) tends to be more unstable than does that of the older.

It seemed desirable to determine the immediate cause of these phenomena. In view of Cannon's (2) studies, the conception of an endocrine origin of the differences has a certain plausibility. Against this notion is our observation that we were unable to obtain any but a normal response to carbonate stimulation from the segment from the normal animal after application *in vitro* of extracts of the homologous adrenals, hypophysis or thyroid. The dependence of the normal mode of movement of the intestine on the integrity of Auerbach's plexus as demonstrated by Magnus (4) and the hypothesis that emotional disturbances spread to the vegetative system, in part at least, through the splanchnics, led us to study the effect of stimulating the superior splanchnic. The rats used were all males of the same age and living in the same conditions. The tracings obtained are given in figure 5. The tracing A was made by the isolated segment of an undisurbed rat when stimulated by M/10 sodium carbonate. It is normal. The tracing B was made by the segment from a rat that had been annoyed and shows the characteristic effect of excitement on the response. Tracing C was made bt the segment from an undisturbed rat which had been opened immediately after killing and the splanchnic nerve electrically stimulated before removal of the segment. It is quite evident that in this case the preliminary splanchnic excitation has induced changes in the intestine of a nature that has caused the segment to respond as do segments obtained from young emotionally excited males. Splanchnic irritation is therefore a fourth factor in causing irregularity in response to sodium carbonate stimulation. The unusual height to which the curve rises is noteworthy although no explanation can be offered for the intense response after the preliminary relaxations. This relaxation on the application of the sodium carbonate solution to the duodenal segment isolated after splanchnic stimulation is similar in type to the relaxation of the intestinal segment of the intact animal after splanchnic stimulation alone, as obtained by Bayliss and Starling (5), and is very suggestive.

Of the four factors so far investigated it seems as if the last revealed the path for the expression of the other three. There is at present no reason to suppose that the splanchnic stimulation could so affect the adrenals as to cause a secondary reaction through the mediation of adrenalin on the intestinal segment since there was no vascular circula-

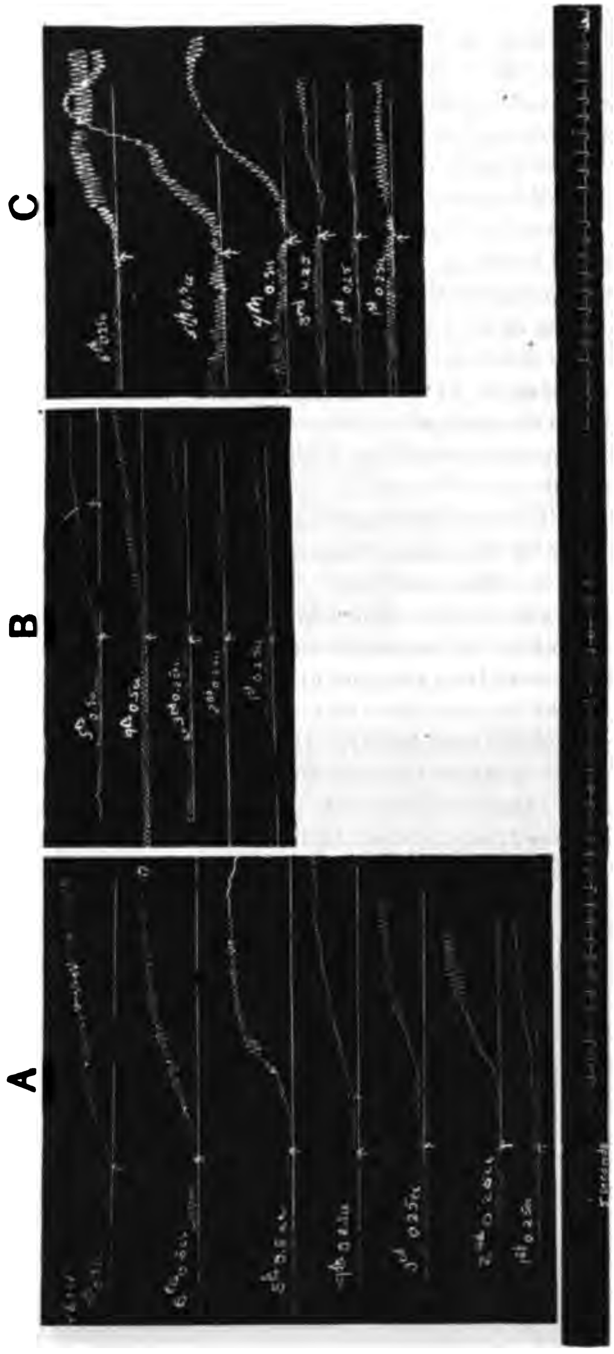

Fig. 5. *A*. Undisturbed normal male rat. Age 80 days. *B*. Disturbed normal male rat. Age 80 days. *C*. Normal male rat electric splanchnic stimulation. Age 80 days.

tion at the time stimulation began. And further, we showed earlier that adrenalin did not modify the response to sodium carbonate. Consequently we are inclined to the opinion that the irregularities of response observed are due to disturbances of the intestinal plexus.

SUMMARY

These experiments demonstrate that the normal response of an isolated duodenal segment of the albino rat to sodium carbonate stimulation is one of shortening or contraction but that certain factors may modify the type of response.

These irregularities of response of the segments from young, healthy male rats are due to a condition of excitement induced in the animal by change of environment or rough handling just before its use for experimental purposes.

The susceptibility of the intestinal segment to such external disturbances is modified by age in that material from rats of two hundred days fails to show the irregularities after excitation.

Female rats are not suitable subjects for general studies of this nature inasmuch as the act of menstruation sets up such changes in the intestinal segment as to cause it to respond in a manner analogous to the segment from young excited male rats.

The electrical stimulation of the splanchnic nerve of a normal undisturbed male rat before the removal of the segment results in the production of a similar type of response to carbonate stimulation as is that obtained from the segment of the excited rat of the same age. This inclines us toward the hypothesis that the irregularities of response here observed are due to disturbances of the intestinal plexus.

CONCLUSION

It is possible to obtain an intestinal segment which will give a uniform and consistent response to sodium carbonate stimulation. The gastric end of a duodenal segment satisfies these requirements when taken from healthy male adult albino rats eighty to two hundred days old some fifteen hours after the last feeding and in which no emotional disturbance has been induced by recent changes of environment or rough handling. Excitement, age, menstruation and electric stimulation of the splanchnic nerves are factors tending to cause changes in the type of response of the segment to sodium carbonate.

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THE ADJUSTMENT OF BLOOD VOLUME AFTER INJECTION OF ISOTONIC SOLUTIONS OF VARIED COMPOSITION[1]

ARTHUR H. SMITH AND LAFAYETTE B. MENDEL

From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven

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Water plays such a large part in the physical regulation of fundamental physiological processes as well as in the chemical transformations in the body, that the lack of suitable amounts or the abnormal distribution of fluid in the body may be correlated with distinctly pathological conditions. Volume changes in the body take place only through the movement of water.

The various types of edema illustrate what may happen when there is an abnormal distribution of water in the body. Besides the edema associated with cardiac and renal diseases we may add another type known as nutritional edema. The etiology of neither of these conditions is clear.

There are also conditions in which water is lost from the body to such an extent that desiccation takes place and the blood becomes exceedingly concentrated. Such a condition prevails in Asiatic cholera in fatal cases of which Rogers (1) has reported a serum loss of 62 per cent. Intravenous injection of isotonic saline solution in such cases is at once followed by diarrhea with the resulting loss of all the added water. In war gas poisoning Underhill (2) has described the marked loss of water from the blood and the movement of water into the lungs.

The mechanism of the loss of heat from the body is largely physical in character. Balcar, Sansum and Woodyatt (3) have reported experiments in which they were able to produce fever at will by intravenous injections of hypertonic glucose solutions provided diuresis ensued. Temperatures of 109°, 111° and 126° were thus obtained in dogs. These authors have suggested that in the body the water may be either "free" or "combined." The "free" water is available for evaporation with the consequent heat loss while the water combined with the colloids of the body is not available for heat regulation. Any condition which increases the "combined" water or decreases the "free" water leads to a rise in temperature due to the absence of proper heat loss. Lussky and Friedstein (4) reported that in cases of pneumonia ending in crisis in infants, there was a decrease in body weight with crisis due to loss of water.

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[1] The data in this paper are taken from a dissertation presented by Arthur H. Smith for the degree of Doctor of Philosophy, Yale University, 1920.

The large amount of water in the cell may aid considerably in maintaining the optimum temperature of the cell, for water has a high specific heat. The large percentage of water in tissues in which oxidation is most intense, may be correlated with this unique property of acting as a heat buffer.

Another evidence of water movement in the body has recently been reported by Weed and McKibben (5). They found that the brain increased in volume after injection of hypotonic salt solutions or distilled water and decreased in volume after hypertonic solutions—these changes being independent of vascular changes and of the cerebrospinal fluid pressure. The remarkable clinical study of Cushing (6) on the alteration of brain volume after administration of hypertonic salt solution per os indicates that the brain is peculiarly sensitive to the movement of water in the tissues.

The question naturally arises as to the physical character of water in the body. By drying blood one finds that it contains nearly 81 per cent of water. But is this water in the form with which we are familiar? In health some of this water is in such a form that it can be given off as vapor in the lungs and can be appropriated by the sweat glands and given off as water. When one considers, however, that in the blood and in the cells there is really a solution of protein and lipoid in aqueous solution of salts, it becomes apparent at once that the physical chemical relationships of water to the other components of the body fluids are exceedingly complex. The hydrophilous colloids probably take up water by adsorption on the internal or disperse phase. This adsorbed water then dissolves in the substance of the particles of the colloid. Large amounts of water are thus taken up by gums, proteins and other emulsoid colloids with the result that swelling takes place.

This imbibition of water is dependent on a variety of conditions such as hydrogen ion concentration and nature of the salt present. It is to the study of some of these conditions on the passage of water out of the circulation that the present investigation is devoted.

In the study of the application of physical laws to the behavior of colloids, much importance has been attached to the early work of Hofmeister and his pupils. Lewith (7) studied the efficiency of various ions in precipitating the serum proteins. The arrangement of his series in the ability to precipitate the proteins was as follows: nitrate<chloride<acetate<sulfate. Hofmeister (8) studied the comparative efficiency of anions in salting out egg globulin and obtained the following series: sulfate>phosphate>acetate>citrate>tartrate>bicarbonate>chromate>nitrate>chlorate. In a later series of experiments (9) he found that the order of efficiency in precipitating gelatin was sulfate>citrate>tartrate>acetate>chloride>nitrate>chlorate. For the precipitation of colloidal iron the series was practically the same. When he studied the swelling of gelatin discs in various solutions, Hofmeister (10) obtained the following order of efficiency: sulfate, tartrate, citrate<acetate<chloride<chlorate, nitrate, bromide, in prompting the swelling of the discs.

Pauli (11) found that different salts raised the coagulation temperature of proteins to different degrees and the order of efficiency was citrate, sulfate<chloride<iodide<acetate<chlorate<nitrate<bromide. Lillie (12) reported that the order of salts in decreasing the osmotic pressure of gelatin and egg albumen was sulfate, tartrate, citrate, phosphate, ferricyanide>fluoride, chloride, nitrate, chlorate>bromide>iodide>sulfocyanate.

Studies of the same series of salts applied to "living" colloids, i.e., to the protoplasm or membrane of cells, have in many cases shown a somewhat similar order of activity. Höber (13) found that the order of anions for stimulating frog muscle was sulfate<chloride<nitrate<bromide<iodide<sulfocyanate. Likewise the order of efficiency of the anions in hemolyzing blood cells in slightly hypertonic solution was sulfate<chloride<bromide<nitrate<iodide. Lillie (14) has pointed out that those salts that are good activators for Arbacia eggs, increase the surface permeability of the egg and the order of efficiency of the salts is chloride<bromide<nitrate<sulfocyanate<iodide. Höber (15) studied the absorption of salts from the small intestine. Sodium chloride was absorbed faster than sodium sulfate and for the series he found chloride>bromide>iodide>sulfate in speed of absorption.

Reviewing the evidence it seems that the sulfate, citrate and tartrate tend always to decrease the permeability, to increase the precipitability of protein, to lessen the imbibition of water, while chloride and acetate assume a middle position and nitrate, bromide, iodide and sulfocyanate tend to increase the permeability, to increase the imbibition of water and to lessen the precipitability of protein. In other words, as Spaeth (16) has pointed out, the order of dispersion of hydrophilous colloids by anions is sulfate<tartrate<citrate<acetate<chloride<chlorate<bromide<iodide<sulfocyanate which is the same order in which these ions produce their deleterious effect on cells.

The method employed in the following series of experiments consisted in the sudden increase of the blood volume to twice the normal volume and the measure of subsequent volume changes by means of hemoglobin determinations. The injected fluid was an isotonic solution of the salt under consideration or, if the toxicity of the substance prohibited, the maximum non-lethal dose made isotonic with sodium chloride.

Healthy rabbits in good nutritive condition were used in all the experiments. The solutions were isotonic with rabbit blood, the osmotic pressure being controlled by freezing point determinations. They were kept at the proper temperature in thermos bottles and injected at 37°C. The injection was made during ether anesthesia through a cannula into the left jugular vein from a graduated cylinder by means of pressure at such a rate that in two minutes a volume of fluid equal to the estimated blood volume of the animal was introduced. In some cases second and third injections were made. In the early experiments the figure 50 cc. per kilo (cf. Boycott, 17) was used as the normal blood volume of the rabbit but later the value $\frac{1}{19.1}$ of the body weight was used.

From a cannula in the right carotid, samples of blood were taken before injection, immediately after injection and thereafter at five-minute intervals for one half hour followed by ten-minute intervals until the end of the experiment. The few drops of blood required for the analysis were caught in depressions of a paraffin plate from which measured volumes were immediately taken for the hemoglobin determinations. In the early part of the work these were made by the method of Haldane (18) using a standard whose oxygen capacity was determined by the method of Barcroft (19). The hemoglobin determinations made later were done by the method of Cohen and Smith (20).

The bladder of the rabbit was emptied by squeezing before the experiment and all of the urine voided during the experiment and that remaining in the bladder on autopsy was collected and measured. At the conclusion of the experiment the animal was killed and autopsy made.

The term relative blood volume is used to mean the ratio of the hemoglobin percentage after the injection to that before the injection. The method of calculating it is as follows: assuming the original volume to be 100, if the hemoglobin value before the injection is 80 and after it is 50, the relative blood volume after injection is $\frac{80}{50} \times 100 = 160$. These figures for relative blood volume, then, are really the percentage of the normal blood volume based on the hemoglobin content.

The use of hemoglobin determinations as an index to the blood volume changes requires special consideration. This is a valid index only if, under the conditions of the experiment, there is a negligible variation in the erythrocyte count due to extrusion of new red cells into the blood stream. Increases in the red count and hemoglobin have been reported in a variety of conditions. Muscular contraction (21), exercise with perspiration (22), increase in blood pressure (23), and decrease in atmospheric pressure (24), have all been reported as conditions under which there is an increased red count and hemoglobin. Lamson (25) has investigated the factors producing polycythemia. He found that in some cases hemorrhage will produce an increased blood count shortly after the blood is lost.

TABLE 1

Comparison of variation in hemoglobin with that of total solids before and after injection of isotonic salt solution

	BEFORE INJECTION	AFTER INJECTION	REDUCTION
	per cent	*per cent*	*per cent*
Experiment 30			
Hemoglobin	90.0	73.0	81.0
Solids	16.0	13.3	83.0
Chlorides of whole blood	0.42	0.36	86.0
Experiment 46			
Hemoglobin	67.0	56.0	16.4
Solids	13.8	11.9	13.4
Experiment 48			
Hemoglobin	75.0	64.0	14.6
Solids	14.7	12.9	12.2

TABLE 2

The effect of repeated small hemorrhages on hemoglobin percentage

TIME INTERVAL FOR REMOVAL OF SAMPLES CA. 1-2 CC.	EXPERIMENT NUMBER		
	40	50	51
minutes			
Normal	67	89	65
5	65	90	65
10	62	90	65
15	60	90	66
20	59	90	65
25	59	90	66
30	59	90	67
40	59	89	66
50	59	90	65
60	59	89	65

Among other factors he mentions emotional excitement and anesthesia. If these factors were to play a part in our experiments the validity of the results obtained might be questioned.

Lamson quotes figures illustrating the increase in red count in a cat after fright. The degree of polycythemia is exceedingly variable. He also states that immedi-

ately after anesthesia with ether the red count is above normal but that it gradually falls. It is conceivable that there may be an increase in red count without an increase in hemoglobin.

Experiments were carried out to determine whether or not the hemoglobin could be used as an index to blood volume under the conditions of the procedure outlined above and also whether the hemoglobin percentage changed as a result of excitement and anesthesia in these experiments. The results (tables 1 and 2) show that not only did anesthesia, fright and withdrawal of successive small volumes of blood not change the hemoglobin percentage, but that the hemoglobin percentage varied in a parallel manner with the total solids. It seems reasonably certain, then, that under the experimental conditions outlined, the hemoglobin is a valid and useful index to blood volume changes.

Inasmuch as glycosuria has been observed following intravenous injections of salt solutions, it seemed of importance to determine whether or not there was hyperglycemia which, of itself, might exert some influence on the dilution of the blood. In the present experiments there was no glycosuria following the injections except with colloidal silver. In one experiment (exper. 24) when sodium sulfocyanate was injected, the blood sugar was 0.075 per cent before anesthesia, 0.112 per cent after anesthesia and 0.209 per cent one half hour after the injection. At this time the relative blood volume had returned to normal so that it may be said that such hyperglycemia as follows intravenous injection of salt solutions, exerts no appreciable effect on blood volume. This point needs, however, further investigation.

Experiments with salt solutions. The following solutions were used: Sodium chloride 0.98 per cent, sodium acetate 1.23 per cent, sodium bromide, 1.8 per cent, sodium nitrate 1.5 per cent, sodium sulfocyanate 1.36 per cent, sodium sulfate 2.0 per cent, sodium tartrate 1.5 per cent in 0.45 per cent sodium chloride and sodium citrate 0.26 per cent in 0.9 per cent sodium chloride. These solutions were isotonic with rabbits blood. The injection was rapid—a volume equal to the blood volume being introduced in two minutes. In the case of the chloride, bromide and sulfate in some of the experiments, the respiration was slower and more labored during the injection. In the case of tartrate the respiration was quickened. With the citrate there were muscular spasms.

From table 3 it will be seen that the salts differ somewhat in their influence on the passage of water out of the circulation. With citrate, tartrate and sulfate the relative blood volume stayed above normal longer than in any other case; with the chloride the return to normal was more rapid while with the bromide, acetate and nitrate the return to normal blood volume was most rapid. Except in the case of the citrate, tartrate and sulfate, however, the differences were negligible.

In all of these experiments the larger part of the injected fluid disappears within the first five minutes and a considerable part of it leaves the circulation during the injection (17). Immediately after the injection there is an enormous increase in the filtration pressure in the capillaries. This increased pressure accounts for the rough parallelism in volume changes between all the salts within the first five minutes after injection.

TABLE 3

Regulation of blood volume after injection of solutions of various sodium salts; average results of all experiments; relative blood volume

TIME INTERVAL	SODIUM SALT							
	Acetate	Nitrate	Sulfo-cyanate	Bromide	Chloride	Tartrate	Sulfate	Titrate
minutes								
Normal	100	100	100	100	100	100	100	100
Immediately	164	166	158	142	141	148	148	137
5	116	130	128	119	118	123	124	119
10	108	118	117	111	114	118	116	120
15	104	108	111	109	112	115	112	116
20	104	108	107	105	109	110	112	115
25	104	106	106	104	106	110		114
30	101	103	104	104	105	111	112	114
40	100	100	102	101	102	111	112	114
50		100	102		102	111	109	
60							107	

The differences in behavior between the citrate, tartrate and sulfate and the rest of the salts became apparent later in the experiment, i.e., twenty-five or thirty minutes after the injection. The effect of the given salt on the diffusion of fluid is then seen. On the basis of the present experiments the salts can be arranged in the order of their effect in permitting the passage of fluid out of the circulation, as follows: —acetate, nitrate, sulfocyanate, bromide > chloride > tartrate, sulfate, citrate.

The physical chemical systems in the blood vessels immediately after introducing the injection fluid are very complex. In the blood the plasma proteins, the red corpuscles and lipoids occur suspended in, or dissolved in, a water solution of various inorganic salts. It is obvious that the injection fluid will first react with the substances in the blood insofar as it is capable of producing any change in the equilibrium there. Since proteins are ionized and obey the law of mass action (26), (27) a chemical readjustment in the blood stream after the injection of the salt solutions may be expected. Hence the character of the fluid thus established in this newly constituted system may be sufficient to account for the differences of rate with which fluid leaves the circulation after the injections of various salt solutions.

Again, although we were very careful to have the solutions isotonic when injected, it does not follow that the osmotic conditions in the blood stream remain unchanged after the addition of the salts. There may have been some degree of ion-colloid readjustment or even double decomposition between inorganic salts which might change the osmotic pressure of the blood temporarily. The calcium precipitants, especially, might act in this way.

A third possible factor in regulating the speed with which the added fluid leaves the circulation is the "permeability" of the membrane forming the capillary wall. It is obvious that most of the investigations of the permeability of isolated plant and animal cells can not be applied to the capillary endothelial membrane except in a very general way, for the proteins and especially the salts of the plasma constitute, as can be inferred from the above discussion, a very effective system for maintaining not only a constant reaction but also a constant balance of salts and colloids. It is because of this regulating mechanism that the difference between the salts used in these experiments was so small when judged by their effect on the movement of fluid out of the circulation. The sulfate, tartrate and citrate action was more pronounced because of their double effect: they may have reduced the water-holding capacity of serum proteins either through formation of less ionized combinations or through purely physical means; and secondly, they may have decreased the permeability of the capillary membranes so that the free movement of surplus fluid out of the circulation observed with the chloride, for instance, was interfered with. It is possible that the combination of the above mentioned factors resulted in the diminished speed of return of the augmented blood volume observed after injection of solutions containing sulfate, tartrate or citrate.

The analogy between the action of the various salts on the capillary membranes and on other membranes is suggestive. Magnesium sulfate, sodium sulfate, magnesium citrate and sodium potassium tartrate, saline cathartics containing anions which appear at one end of our series

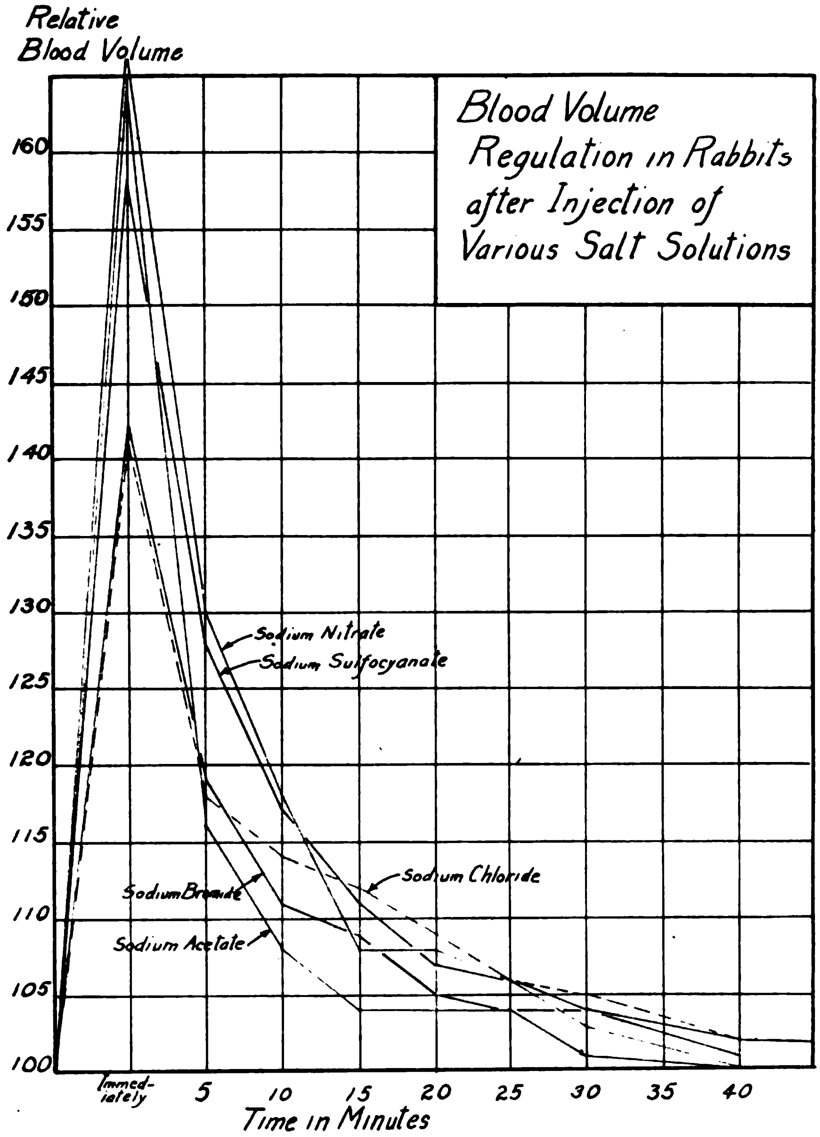
Relative Blood Volume
Blood Volume Regulation in Rabbits after Injection of Various Salt Solutions
160
155
150
145
140
135
130
125
120
115
110
105
100
Immediately
5
10
15
20
25
30
35
40
Time in Minutes
Sodium Nitrate
Sodium Sulfocyanate
Sodium Chloride
Sodium Bromide
Sodium Acetate

Chart 1

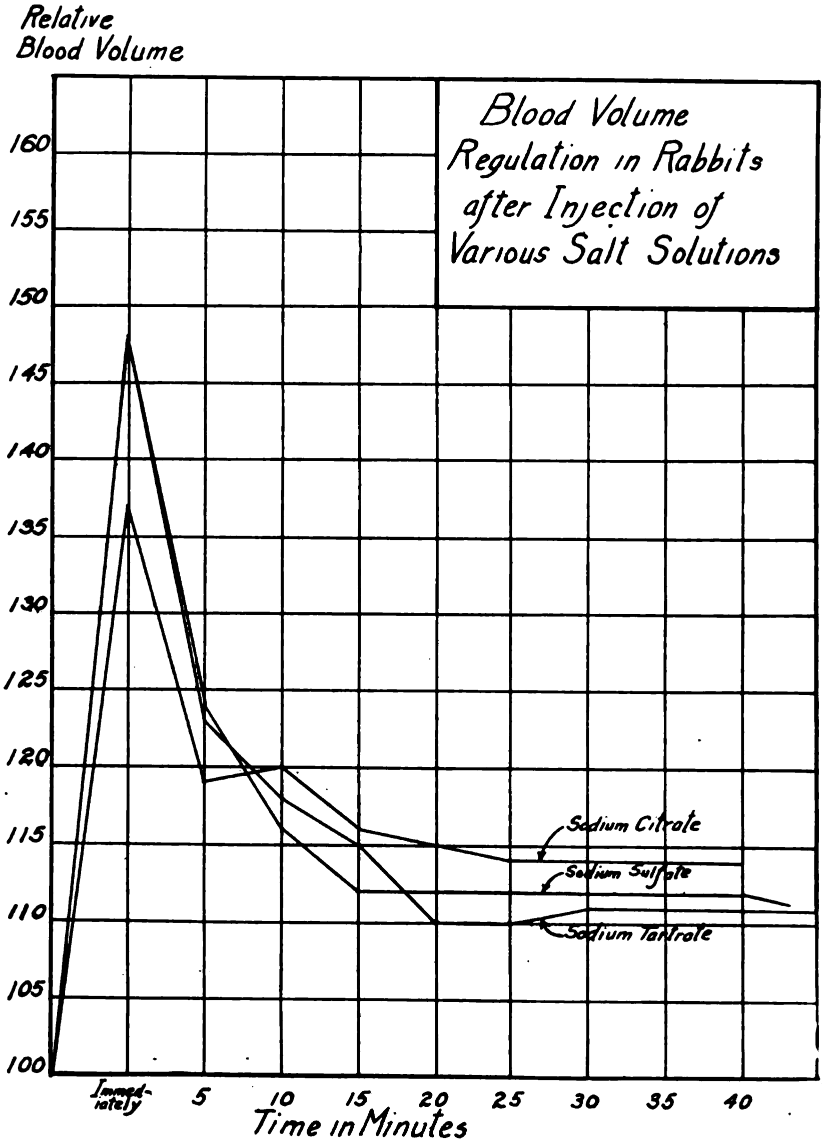
Relative Blood Volume
Blood Volume Regulation in Rabbits after Injection of Various Salt Solutions
160
155
150
145
140
135
130
125
120
115
110
105
100
Sodium Citrate
Sodium Sulfate
Sodium Tartrate
Immediately
5
10
15
20
25
30
35
40
Time in Minutes

Chart 2

do not easily diffuse through the gut wall into the blood stream, and so they act osmotically to draw water from the blood stream to flush the intestine. Goldschmidt and Dayton (28) have shown that this is precisely what happens in the colon. There was free passage of water with practically no diffusion of sulfate in their experiments. Höber (15) has studied the absorption of salts from the small intestine and finds that the anions of the salts arrange themselves in the same order as in the present experiments, sulfate being absorbed least rapidly and chloride most rapidly. Wallace and Cushny (29), studying the intestinal absorption of saline cathartics, found that the salts arrange themselves in order of the speed of absorption as follows:—chloride, bromide, iodide > sulfate > phosphate, tartrate, citrate.

The effect of calcium. Calcium was studied in these experiments first because, as Loeb (30) and Osterhout (31) have pointed out, it exhibits an antagonism toward sodium and secondly because of its alleged action on the permeability of membranes. In experiments on the prevention of pleural exudates in dogs, Chiari and Januschke (32) reported that calcium injected subcutaneously inhibited the transudation after treatments which in control dogs caused a copious amount of fluid. The whole scheme of experimentation was rather severe in this investigation. The appearance of icterus after the injection of fluorescin was found by Rosenow (33) to be distinctly inhibited by calcium chloride.

Small amounts of concentrated sodium chloride injected intravenously in rabbits elicit a hyperglycemia which is prevented by adding calcium chloride to the salt solution (34). Slow injections of large volumes of sixth molecular sodium chloride into rabbits produce a glycosuria which is prevented by the addition of three-eighths molecular calcium chloride in the ratio 975 cc. NaCl to 25 cc. $CaCl_2$ (35). MacCallum (36) found that saline purgatives injected intravenously produced active peristalsis but that the action was inhibited by calcium chloride. Calcium antagonizes not only sodium but also magnesium (37) and potassium (38), (39), (40), (41).

In the present experiments calcium chloride was injected with sodium chloride in isotonic solution to ascertain whether or not the calcium would hinder the movement of fluid out of the capillaries. As will be seen from table 4, instead of inhibiting the return to normal relative blood volume, the presence of the calcium seemed, if anything, to hasten the outward passage of the injected fluid. Yanagawa (42) had reported that calcium chloride does not reduce the permeability of the capillaries under normal conditions.

In the second injection of experiment 31, 0.25 gram calcium chloride dissolved in isotonic sucrose solution was injected and the animal died with tetanic convulsions. The left ventricle was in strong contraction and there was a clot in the right auricle. When a higher concentration of calcium chloride in sodium chloride solution was injected there were no untoward effects. This seems to indicate that the sodium exerted an antagonism not shown by the sucrose.

TABLE 4

Regulation of blood volume after injection of sodium chloride containing calcium chloride, relative blood volume

TIME INTERVAL	EXPERIMENT NUMBER		
	32 (First injection)	32 (Second injection)	33 (First injection)
	250 cc. 0.9 per cent NaCl 6.4 cc. ½M $CaCl_2$	250 cc. 0.9 per cent NaCl 12.8 cc. ½M $CaCl_2$	250 cc. 0.85 per cent NaCl 20 cc. ½M $CaCl_2$
minutes			
Normal	100	100	100
Immediately	123	141	134
5	108	113	110
10	102	105	100
15	100	105	100
20	100	100	100
25	100	100	100
30			
40			
50			
60			

The effect of colloidal silver. Colloidal silver is a suspensoid colloid while the blood proteins are emulsoid colloids. Spiro (43) has reported that the imbibition of water by gelatin is accelerated by the presence of colloidal iron oxide. The present experiments were carried out to determine the effect of the interaction of the two types of colloids upon the movement of water out of the circulation. The technic was the same as used before in the present experiments. The silver preparation used (Solargentum, Squibb)[2] is said to contain about 20 per cent silver It was dissolved in 0.97 per cent sodium chloride solution and in three cases the dosage was 50, 100 and 200 mgm. respectively dissolved in 100 cc. of the salt solution. It caused no depression of the freezing point and gave a golden brown solution which did not interfere with the hemoglobin determinations.

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[2] This preparation was kindly furnished through Dr. I. F. Harris by E. R. Squibb & Sons.

Table 5 shows the results of injecting colloidal silver solutions. When the amount injected was 50 or 200 mgm. there was no appreciable effect on the return to normal of the relative blood volume. When 100 mgm. were injected the relative blood volume remained above normal. This injection was, however, the second dose of colloidal silver that rabbit had received. It appears that there may be a cumulative effect of the colloidal silver solution. There was glycosuria in all cases after the injection of colloidal silver. This point will be investigated further.

TABLE 5

Regulation of blood volume after injection of colloidal silver solution (Solargentum Squibb); relative blood volume

TIME INTERVAL	EXPERIMENT NUMBER		
	44 (First injection)	44 (Second injection)	45
	100 cc. 0.97 per cent NaCl 50 mgm. silver	100 cc. 0.97 per cent NaCl 100 mgm. silver	100 cc. 0.97 per cent NaCl 200 mgm. silver
minutes			
Normal	100	100	100
Immediately	143	132	142
5	122	118	129
10	106	113	106
15	104	113	106
20	106		101
25	104	111	101
30	104	111	101
40	104	111	100
50			
60			

The effect of acacia. Since varying accounts of the value of acacia in shock have been reported, it seemed of interest to try its effect in normal animals under the conditions of our experiments. In circulatory shock saline solution or Ringer's solution given intravenously leaves the circulation so rapidly that it helps little. Bayliss (44), (45), (46) introduced the use of gum acacia and has been its foremost champion. The value of acacia lies in the fact that it possesses not only viscosity but also a small osmotic pressure thus simulating the plasma proteins. He recommends the intravenous use of 7 per cent acacia in 0.9 per cent sodium chloride solution in the treatment of shock on the theory that if the blood pressure and aeration are kept up acidosis need not be feared.

A study of the blood volume regulation in normal animals after the injection of acacia was made in the present investigation according to the technic already described, a solution of 7 per cent acacia in 0.9 per cent sodium chloride ($\Delta = 0.59$) solution being used. The Δ due to the acacia alone was 0.07.

Table 6 shows that considerable fluid passed out of the circulation during the injection. The acacia maintained the augmented blood volume in a way not observed with any other substance used in these experiments. It held the relative blood volume one-third above normal for more than an hour.

TABLE 6

Regulation of blood volume after injection of acacia-sodium chloride solution; relative blood volume

TIME INTERVAL	EXPERIMENT NUMBER		
	33 (Second injection)	52	53
minutes			
Normal	100	100	100
Immediately	153	141	144
5		141	137
10	146	141	137
15	146	141	137
20		141	137
25	136	141	137
30	134	141	134
40	133	141	131
50	133	135	131
60	133	129	131

Moore (47) attributes the value of acacia to the restoration of the hydrophilous colloids to the blood. That it exerts a small persistent osmotic pressure and thus maintains the blood volume has been asserted by Gasser, Erlanger and Meek (48). Kruse (49), however, has suggested that the value of acacia in maintaining blood volume may be due to its adsorption on the capillary walls, whereby the exit of the fluid from the vessels is impeded.

The effect of acid. The effect of acid on the swelling of colloids and on the permeability of membranes has been widely studied. Spiro (43) reported that $\frac{M}{100}$ hydrochloric acid caused gelatin discs to swell more than did water, while Chiari (50) showed that increased swelling was obtained when carbon dioxide was present in conductivity water. Fischer (51) demonstrated the swelling of muscle in acid. Osterhout (52) studying diffusion, found that acid first decreased the permeability of the cell membrane but later increased it. Harvey (53) showed that all acids excepting benzoic and salicylic encounter resistance to diffusion at the surface of the living cell.

It might appear a priori that injection of acid would increase the water-holding capacity of the plasma colloids or would decrease the permeability of the capillary membrane to such an extent that the passage of fluid out of the circulation after injection would be inhibited. On the other hand, the body has such remarkable ability to take care of increased acid and to maintain its constant reaction (45) that it would appear problematical whether or not one could introduce enough acid to change the physical chemical equilibria without killing the animal.

TABLE 7

Regulation of blood volume after injection of hydrochloric acid-sodium chloride solutions; relative blood volume

TIME INTERVAL	EXPERIMENT NUMBER			
	34	38 (Second injection)	54	56
	$\frac{M}{20}$ HCl in 0.7 per cent NaCl	$\frac{M}{15}$ HCl in 0.6 per cent NaCl	$\frac{M}{15}$ HCl in 0.6 per cent NaCl	$\frac{M}{15}$ HCl in 0.6 per cent NaCl
minutes				
Normal	100	100	100	100
Immediately	117	124	121	138
5	111	111	108	115
10	108	108		109
15	104	105	104	103
20	104	102	108	103
25	102		104	101
30	101	108	104	103
40		105	102	101
50			104	101
60			104	103

To test this point with reference to changes within the blood vessels, $\frac{M}{20}$ and $\frac{M}{15}$ hydrochloric acid made isotonic with 0.7 per cent and 0.6 per cent sodium chloride respectively were injected as in experiments already recorded. $\frac{M}{15}$ acid seemed to be the limit of tolerance under the conditions of the experiment. Several of the rabbits died during the injection and on autopsy showed pulmonary edema. During infusion there were in every case dyspnea and spasmodic contractions of the voluntary muscles.

From table 7 it will be seen that in the concentrations used hydrochloric acid failed to decrease the rate of passage of fluid out of the blood vessels. The relative blood volume returned to normal as rapidly when acid-sodium chloride solutions were used as with the sodium chloride alone.

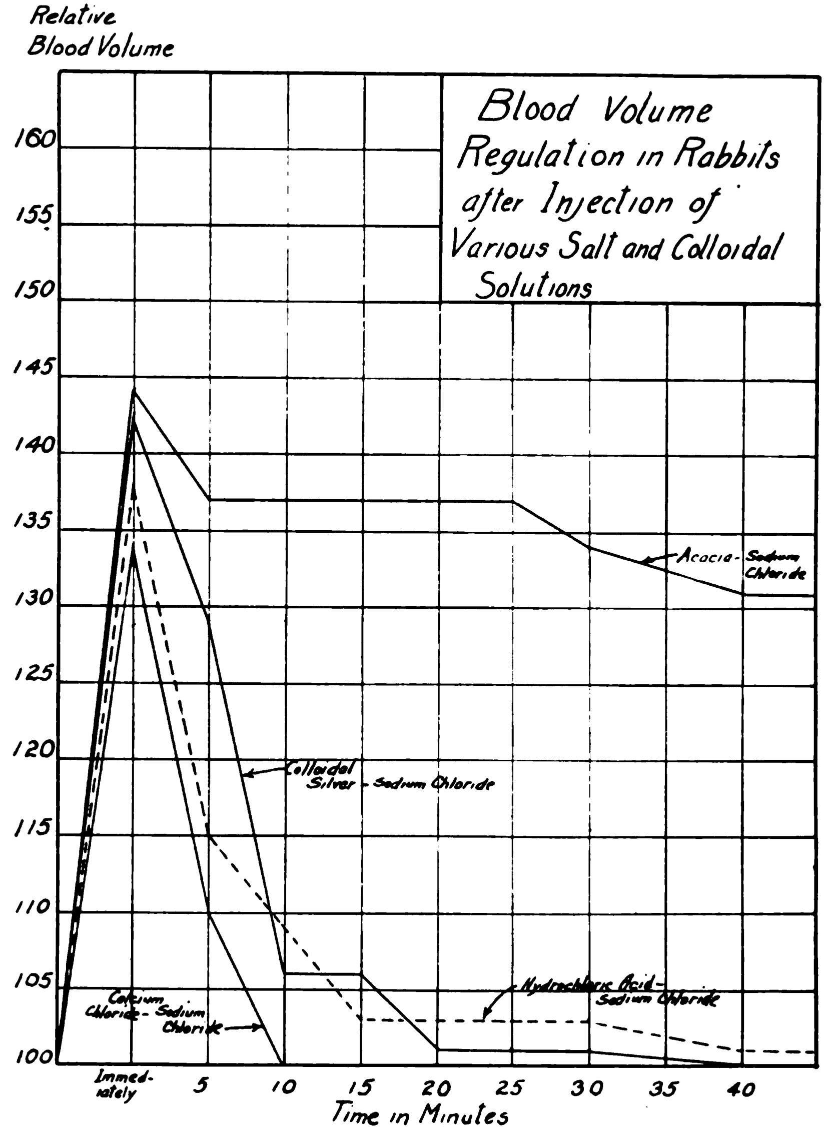
Relative Blood Volume
Blood Volume Regulation in Rabbits after Injection of Various Salt and Colloidal Solutions
160
155
150
145
140
135
130
125
120
115
110
105
100
Acacia-Sodium Chloride
Colloidal Silver - Sodium Chloride
Hydrochloric Acid-Sodium Chloride
Calcium Chloride - Sodium Chloride
Immed-iately
5
10
15
20
25
30
35
40
Time in Minutes

Chart 3

The effect of sucrose. To determine the effect of a crystalline non-electrolyte on the removal of fluid added to the blood 10.9 per cent sucrose solution (Δ = 0.59) was injected in one experiment. From table 8 it will be seen that the relative blood volume returned to normal as rapidly as with saline solution.

TABLE 8

Regulation of blood volume after injection of sucrose solution; relative blood volume

TIME INTERVAL	EXPERIMENT NUMBER 31
minutes	
Normal	100
Immediately	158
5	146
10	119
15	109
20	104
25	101
30	100
40	100
50	
60	

Fate of the injected fluid. From the preceding experiments it is evident that with the exception of the citrate, tartrate, sulfate and acacia none of the substances injected decreased the rate of loss of fluid from the circulation as judged by the variation in blood volume. In the majority of cases a volume of fluid equal to the blood volume diffused out of the circulation in less than one half-hour. In speculating upon, and, if possible, determining the fate of this fluid, attention is directed to the possible paths of elimination of this excess of volume. It may be excreted through the kidneys, it may accumulate as edema fluid, it may form serous exudates, it may pass into the tissue spaces or it may be excreted into the gastro-intestinal tract.

Haldane and Priestley (54) have shown in a striking manner that copious water drinking results in a large urine output. The effect of water drinking upon the circulation must be analogous to that produced by the injection of isotonic solutions in our experiments. Magnus (55), (56) concluded that the cause of the diuretic action of salts lies in the composition of the blood and not in the increased capillary pressure. He found that sulfate solutions of the same osmotic pressure as chloride solutions produced greater diuresis.

TABLE 9

Urine volume as percentage of injected solution

SOLUTION	EXPERIMENT NUMBER	PER CENT	AVERAGE
Sodium sulfate	2	55	
	7	64	
	20	51	
	27	110	70
Sodium nitrate	4	28	
	5	66	
	17	63	
	28	22	
	30	37	43
Colloidal silver	44	16	
	45	29	23
Sodium citrate	36	28	
	37	21	
	38	14	21
Sodium sulfocyanate	3	27	
	14	17	
	23	13	19
Sodium acetate	15	15	
	22	23	19
Calcium chloride-sodium chloride	32	14	
	33	20	17
Sodium tartrate	46	26	
	48	8	
	48	7	13
Hydrochloric acid-sodium chloride	34	10	
	54	16	
	55	8	11
Sodium chloride	9	6	
	11	12	
	18	8	9
Sodium bromide	10	8	
	12	8	
	13	12	9

Table 9 shows the average figures for urine volumes excreted during the various experiments and calculated as percentages of the injected volume. Sulfate and nitrate induced the largest secretion of urine observed with any of the salts or substances investigated. This corresponds with the statements in the literature on the comparative diuretic effect of these salts. However, even in the sulfate experiments where the salt caused the greatest diuresis the urine volume did not account for the fluid which had left the circulation.

The nature of edema and the factors involved in its production have long been matters of discussion. Cohnheim and Lichtenheim (57) injected salt solution until they had given 46 per cent of its weight to a rabbit and 64 per cent to a dog yet they failed to observe subcutaneous edema. On infusing salt solution after arsenic poisoning, Magnus (58) produced edema although in normal animals plethora elicited no edema. It is evident from the literature that hydremic plethora will not produce the edema which is characteristic of nephritis. In the present experiments no patent edema was observed in any of the animals.

The pleural and peritoneal cavities offer considerable free space for the accumulation of pathological fluids. After injection of saline solution Cohnheim and Lichtenheim (57) found fluid in the peritoneal cavity but the pleural cavity was dry. Of the fluid injected into rabbits, Dastre and Loye (59) found 75 per cent in the tissue spaces and serous cavities. In the present experiments the pleural cavity was, as a rule, normal in appearance. The peritoneal cavity usually contained fluid but there was never more than 5 cc. of transudate which usually contained protein and the salt injected and would clot. It is certain that the fluid in the peritoneal cavity accounted for very little of that which diffused out of the blood vessels.

The indefinite area known as "tissue spaces" constitutes a reservoir of fluid in the animal body. After hemorrhage the volume of blood is brought back toward normal by the passage of lymph from the muscle and other tissues into the circulation. Hypertonic solutions draw part of the fluid which renders them isotonic from the tissue spaces. Engels (60) reported that 68 per cent of the fluid injected into a dog was contained in the muscles. In the present investigation experiments were carried out to determine whether or not the amount of water contained in the muscle was demonstrably increased. From table 10 it will be seen that the percentage of water did not vary after the injection. This was rather surprising after the statements made in the literature. All of the older experiments dealt with slow infusions extending over a long period of time. Perhaps the manner in which we carried out the experiment altered the distribution of the fluid.

The excretory function of the small intestine has been given considerable attention in the literature. In the course of their experiments on hydremic plethora, Cohnheim and Lichtenheim (57) observed that the stomach and intestine became swollen. Albu (61) reported that after injection the intestinal contents became a thin paste. J. B. MacCallum (62) injected large volumes of normal saline solution intravenously; and from a cannula tied in the intestine he obtained fluid which amounted to 15 per cent, 9 per cent and 10 per cent of the injected volume in three different experiments.

TABLE 10

The water content of muscles before and after injection of isotonic salt solutions

	GASTROCNEMIUS	TIBIALIS ANTICUS
	per cent	*per cent*
Experiment 41		
Before injection	78.0	77.8
After injection	77.5	77.5
Experiment 42		
Before injection	76.0	76.5
After injection	76.2	76.2
Experiment 43		
Before injection	79.5	79.5
After injection	79.3	79.4

In the present experiments abnormal distention was repeatedly noticed which, on autopsy, was seen to be due to the injection of the stomach, small intestine and cecum. The mass of material in these organs was a thin paste and on section fluid streamed from the stomach. These observations indicate that, under the conditions of our experiments, some fluid was excreted into the gastro-intestinal tract.

From the above discussion it appears that the part of the injected fluid which diffused out of the blood stream in these experiments can be accounted for in the urine, in the exudations into the serous cavities and in the excretion into the intestinal tract. In these experiments there was, however, no trace of edema nor was there detectable increase in the water content of the skeletal muscles.

SUMMARY

When isotonic solutions of the acetate, nitrate, sulfocyanate, bromide, chloride, tartrate, sulfate or citrate of sodium are injected intravenously at such a rate that a volume equal to the estimated blood volume is introduced in two minutes, the rate at which the added fluid escapes from the circulation, as measured by the relative blood volume at successive subsequent intervals, is decreased to a slight extent by the sulfate, tartrate and citrate.

When calcium chloride, hydrochloric acid or colloidal silver was dissolved in sodium chloride solution and was injected intravenously there was no alteration of the rate of return to normal blood volume.

When acacia-sodium chloride solution was used there was a marked and long sustained increase in the relative blood volume.

Sucrose in isotonic solution did not delay the passage of fluid from the blood vessels.

The fluid which leaves the circulation in the restoration of blood volume after the injection could not be accounted for by the passage into the muscles or by edema fluid. The volume of urine, exudate into the serous cavities and the excretion into the intestine and stomach probably are concerned in the disposal of the fluid leaving the circulation.

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ANTAGONISM OF INHIBITORY ACTION OF ADRENALIN AND DEPRESSION OF CARDIAC VAGUS BY A CONSTITUENT OF CERTAIN TISSUE EXTRACTS

J. B. COLLIP

From the Department of Biochemistry and Physiology of the University of Alberta, Edmonton, Canada

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INTRODUCTION

The presence of the so-called "peristaltic hormones" in various tissues has frequently been demonstrated. Enriquez and Hallian (1) found that extracts of the gastric and intestinal mucosa contained a powerful stimulant for the intestinal musculature. Fawcett, Rahe, Hackett and Rogers (2) found that protein-free aqueous extracts of the liver, pancreas and spleen, and of the pituitary, pineal, thyroid, parathyroid, thymus and adrenal glands caused a characteristic stimulatory effect upon the unstriated muscle fibers of the cat's uterus. This stimulatory effect they found to be antagonized by adrenalin. Abel, Pincoffs and Rouiller (3) have prepared from the intestinal mucosa of the pig a water-soluble powder which showed great physiological activity in stimulating an intestinal or uterine strip or in causing the pancreas to secrete freely.

Stern and Rothlin (4) have recently shown that water extracts of liver, kidney, thyroid, lung, muscle, thymus, bone-marrow, bile and blood, contain two kinds of substances, one causing contraction of smooth muscle, the other relaxation. The former substances only, they hold, are present in lymph glands, adrenal bodies and spleen. They found that the constrictor substances were soluble in alcohol and insoluble in ether, while the depressor substances were insoluble in alcohol but soluble in ether. Both types of substances resisted boiling, but the former were destroyed by alkali while the latter were not. Matsumoto and Macht (5) found that the vas deferens of laboratory animals responds with contractions only to large doses of gland extracts. Cow (6) has found that the inhibitory action of adrenalin upon the uterus of the guinea pig, rat and non-pregnant cat is reversed by previous treatment with pituitrin.

In view of the fact that associated with shock following severe trauma there is always the possibility of certain constituents of damaged tissue entering the blood stream, thus giving a condition somewhat analogous to the intravenous injection of tissue extract, a further investigation of the effects of extracts of various tissues, prepared in a variety of ways, upon isolated organs was undertaken.

The general observations that aqueous extracts of various tissues contain certain principles which increase the tonus of smooth muscle were confirmed. Certain additional phenomena of considerable interest were manifested in several experiments; it is these latter that will be dealt with subsequently.

EXPERIMENTAL

The action upon isolated mammalian intestine and uterus, and on the heart of the terrapin, of tissue extracts prepared in the following ways was studied. Extracts made by grinding fresh tissue in the mortar with pure sand and normal Ringer-Locke's solution and subsequently centrifuging. Extracts made by boiling fresh tissue, previously macerated, with several volumes of distilled water, filtering and concentrating the filtrate on the water-bath to an aliquot volume of the original tissue used, thus rendering the extract practically isotonic. Extracts made by extracting the water-bath dried, water-soluble fraction with 98 per cent alcohol, filtering and evaporating the filtrate to dryness on the water-bath and taking up the residue in Ringer-Locke's solution. Extracts made from tissue previously dried at 110°C. to 120°C. Extracts made by boiling desiccated glandular tissue supplied by Armour & Company, Chicago, with distilled water in amount equal to the water content of the original tissue, and filtering. The desiccated thyroid body, parathyroid gland, thymus gland, pancreas, testes, corpus luteum and pituitary body (anterior and posterior lobes) were treated in this manner.

The uterus and intestine of the rat were used most extensively in this investigation, but preparations were also made from the guinea pig, rabbit, dog, mouse and cat. Preparations were suspended in normal Ringer-Locke's solution contained in a glass perfusion chamber of 40 cc. capacity which was immersed in a water-bath kept at 38°C. Oxygen was continuously bubbled through the perfusion fluid. Movements of the isolated smooth muscle strip were recorded on the drum by means of a Harvard heart lever. The extracts were brought to body temperature previous to being added to the saline bath. The effect of tissue extracts upon the heart of the terrapin was determined in one of two ways. The brain of the animal was pithed, the heart exposed, and the apex of the ventricle connected by a silk thread to a heart lever. Both vagi were then dissected free in the neck and their response to rapid make and break shocks determined. The extract was then injected directly into the ventricle or else it was added to Ringer-Locke's fluid and the heart perfused by means of a cannula placed in the left abdominal vein, the fluid escaping through the cut aorta. Intravenous injections of certain extracts were also made into dogs to determine the effect upon the cardiac vagus and the response to adrenalin administered by the intravenous route.

RESULTS

It was found, of the tissues investigated—heart, lung, spleen, liver, brain, cord, posterior spinal root ganglia, pancreas, skeletal muscle, testes, small intestine, gastric mucosa, corpus luteum, and thyroid, parathyroid, pituitary (anterior and posterior lobe), and thymus glands—that all contained in greater or less amount substances which caused increased tonus, amplitude and rate of contractions of strips of duodenum, small intestine, and virgin and gravid uterus. The substances were not destroyed by boiling and were soluble in alcohol. Depressor substances were also present but the usual effect of the addition of 1 or 2 cc. of an extract to the Ringer-Locke's solution in the bath was an immediate increase in the tonus of the smooth muscle strip taken from the duodenum, jejunum or ileum. It was more difficult to demonstrate stimulatory action in the case of the uterus owing to the fact that so often excellent tonus and rhythmic contractions were manifest prior to the addition of the extract. In the case of the extract of pancreas the immediate effect upon the duodenum was a decrease in the general tonus which, however, was quickly recovered and definite stimulation was manifested (fig. 1, *a*). Pancreatic juice obtained from a cannula placed in the pancreatic duct of a dog did not have this effect. Again in the case of aqueous extracts of oven-dried tissues studied (brain, cord, posterior root ganglia, duodenum, heart, pancreas, spleen, skeletal muscle) a deviation from the general rule was noticed. The primary effect of such extracts upon the intestinal musculature was typically that of adrenalin (fig. 1, *b*). The primary depression which was caused by the addition of such an extract was, however, followed at once by an increase in the general tonus. These latter extracts had little effect upon the uterus unless used in fairly large amount, in which case decreased tonus resulted (fig. 1, *c*).

It was found that extracts prepared from the heart, pancreas, corpus luteum, pituitary body (both anterior and posterior lobes), testes, and thyroid, parathyroid and thymus glands in addition to causing intense stimulation of the uterus antagonized the inhibitory action of adrenalin on such uteri as are normally inhibited by this latter substance, as in the case of the rat and guinea pig (both virgin and gravid), and the virgin rabbit and dog (figs. 1, *d;* 1, *e;* 2, *a;* 2, *b;* 3, *a*). This phenomenon is strikingly similar to that, recorded by Cow (6), of the reversal of the inhibitory effect of adrenalin on the uterus of certain animals by previous sensitization with pituitary extract. The results of Cow (6) were in part confirmed by the writer. Extracts of tissues studied other than those mentioned above, were not as effective in this regard. Partial antagonism was obtained by extracts of lung, gastric mucosa and liver, but in these instances the amounts of extracts used were relatively quite large. This antagonism of the inhibitory action of adrenalin by extracts of certain tissues was effective only as long as the extract was actually present in the saline bath. The inhibitory response to adrenalin returned in each case after the uterine strip was washed free from the extract (fig. 3, *a*). It was difficult to determine absolutely whether the stimulant action of adrenalin on the gravid uterus of an animal such as the rabbit was antagonized by extracts like that of the spleen, as the tonus produced by the addition of the latter was practically maximal; however, the writer is of the opinion that the stimulatory effect of adrenalin is not antagonized on such uteri as are normally stimulated by it.

The antagonism of the inhibitory action of adrenalin on certain uteri by extracts above noted was not demonstrated in the same decisive manner in the case of the intestinal musculature. Partial antagonism was, however, clearly demonstrated on different occasions by the raising of the threshold for adrenalin inhibition of the duodenum or jejunum of the rat by extract of spleen or heart (fig. 4, *a*).

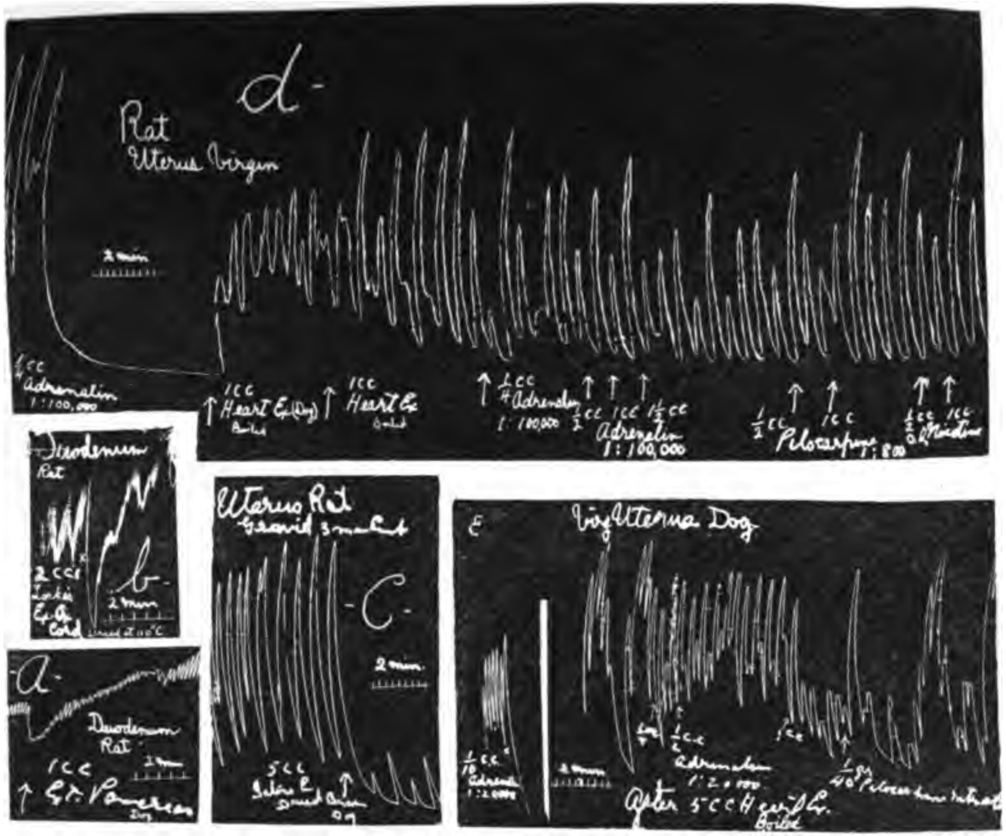

Fig. 1. *a*. Effect of 1 cc. of saline extract of fresh dog's pancreas upon isolated duodenum of the rat.

b. Effect of 2 cc. of extract of ox cord dried at 110°C. upon isolated duodenum of rat.

c. Effect of 5 cc. of extract of brain of dog dried at 110°C. upon isolated gravid uterus of rat.

d and *e*. Antagonism of inhibitory action of adrenalin by heart extract: *d*, non-gravid uterus of rat; *e*, non-gravid uterus of dog.

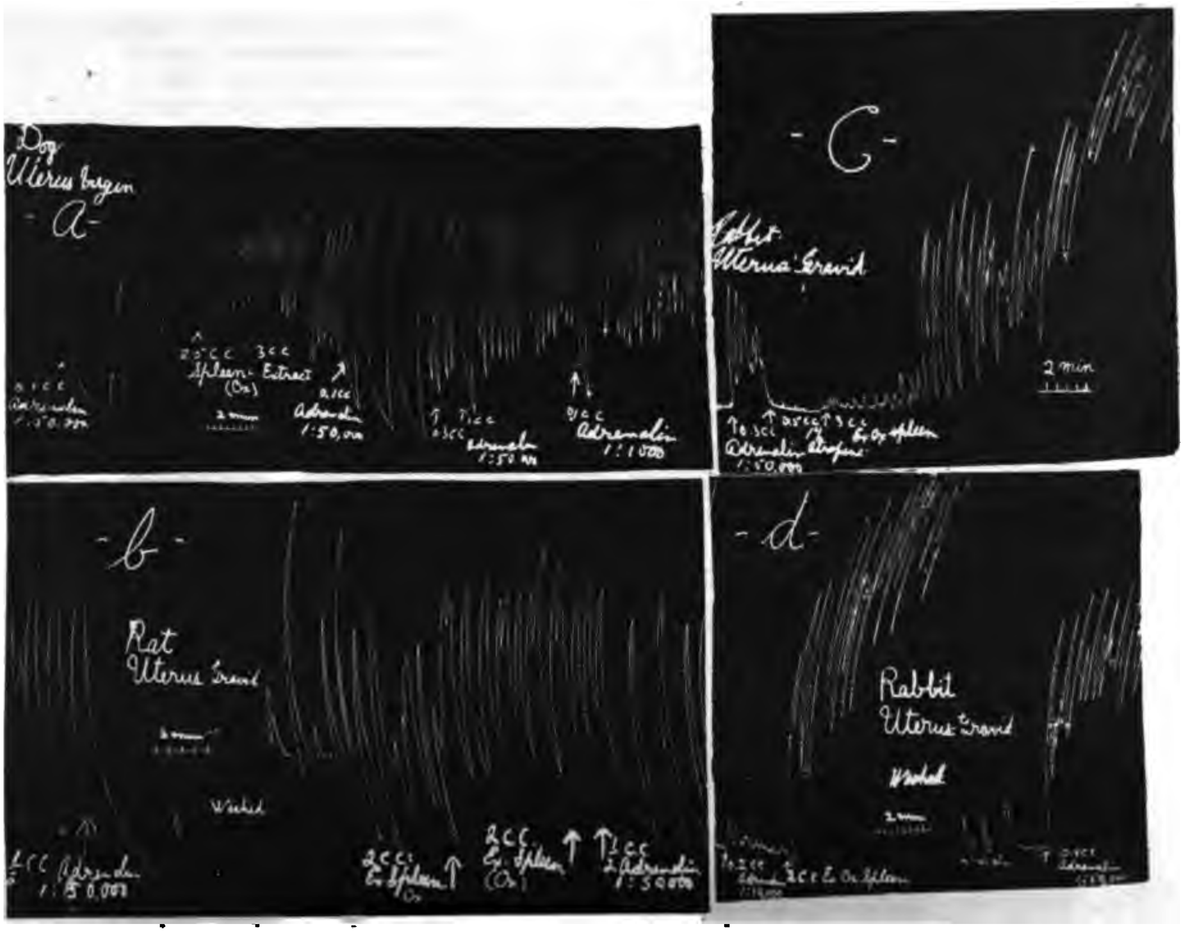

Fig. 2. *a* and *b*. Antagonism of inhibitory action of adrenalin by extract of ox spleen: *a*, non gravid uterus of dog. *b*, gravid uterus of rat.

c. Action of splenic extract upon atropinised gravid uterus of rabbit.

d. Action of adrenalin on gravid uterus of rabbit after splenic extract.

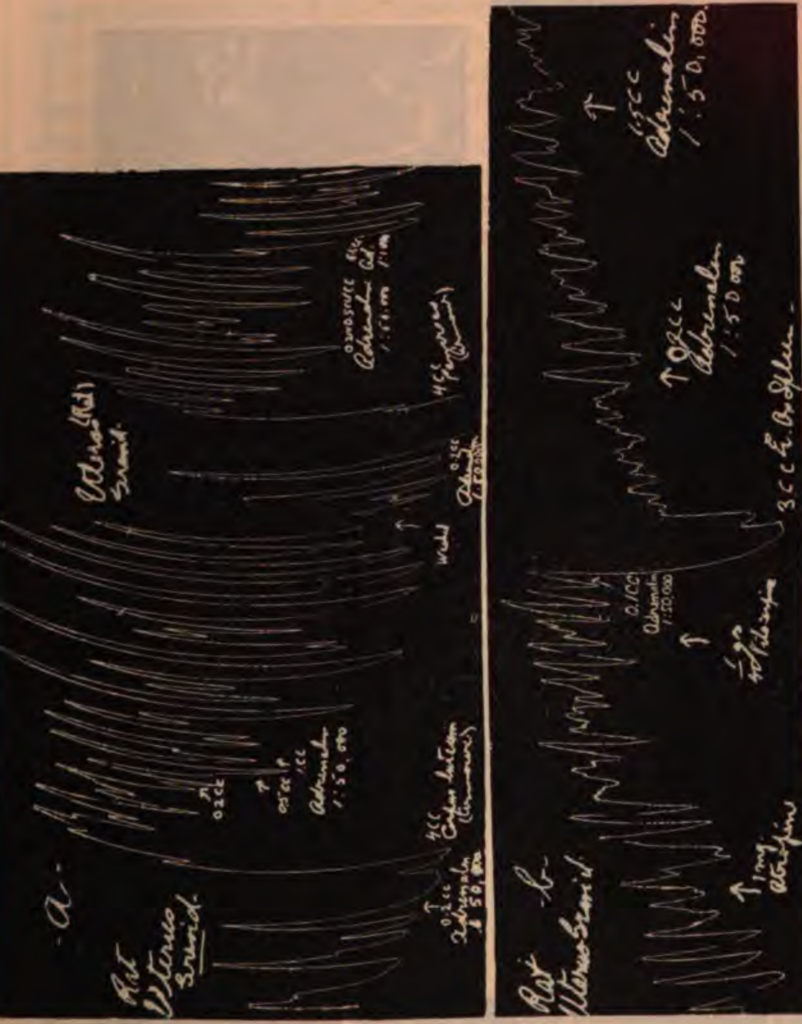

Fig. 3. *a.* Antagonism of the inhibitory action of adrenalin on the gravid uterus of rat by extract of corpus luteum and pancreas (Armour & Co., Chicago).

b. Effect of adrenalin on the gravid uterus of the rat before and after the addition of extract of ox spleen.

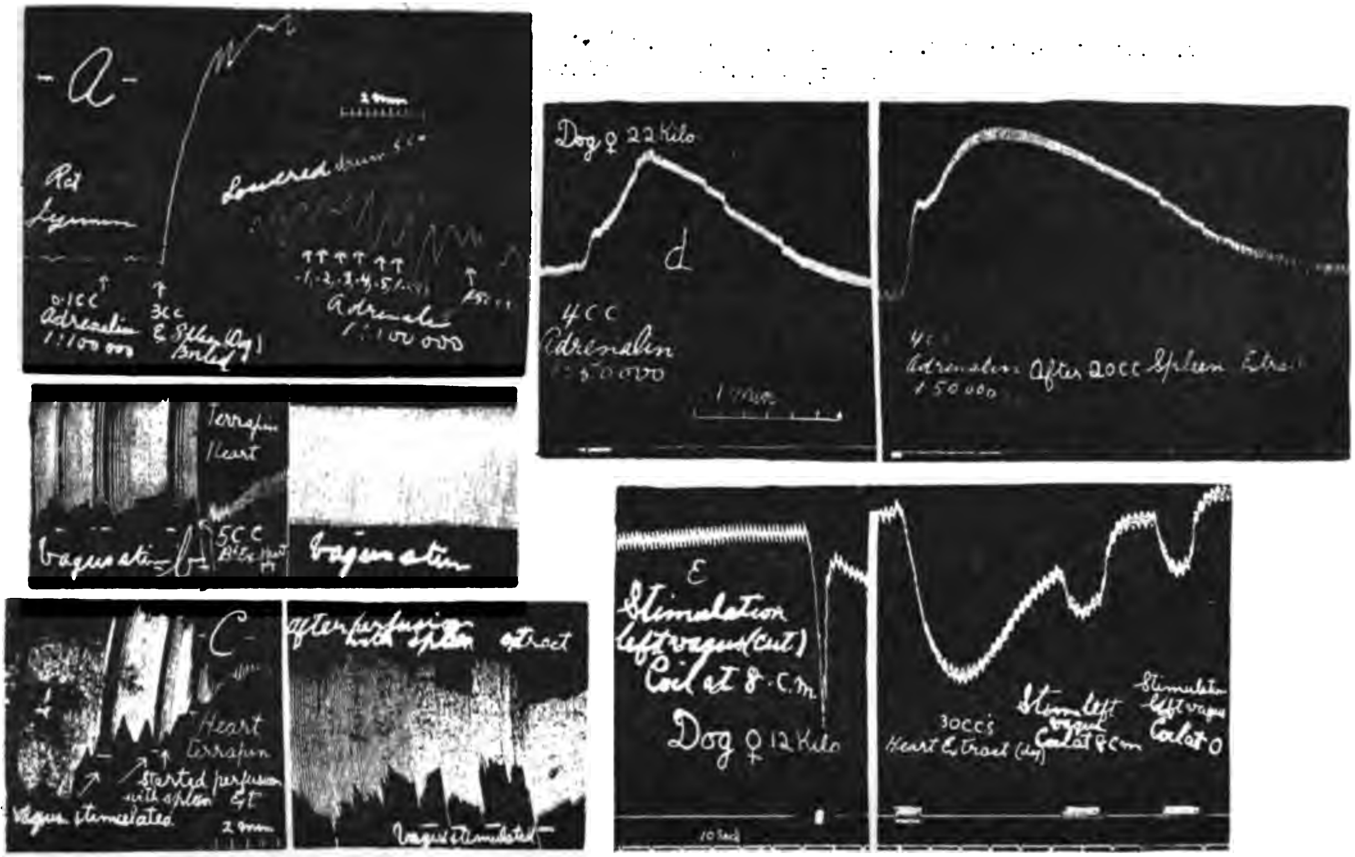

Fig. 4. *a.* Antagonism of the inhibitory action of adrenalin upon jejunal musculature of rat by 3 cc. of boiled extract of dog's spleen.

b and *c.* Complete depression of cardiac vagus of terrapin to electrical stimulation by: *b*, extract of pig's heart; *c*, extract of spleen of ox.

d. Effect of adrenalin before and after extract of spleen.

e. Depression of cardiac vagus of dog by heart extract.

It was found, when splenic extract was injected intravenously into a dog, that the rise in blood pressure produced by a definite dose of adrenalin was slightly augmented, and also the high pressure was longer maintained (fig. 4, *d*). Electrical excitation of the vagus which, prior to the injection of the extract into the ventricle or the perfusion of the same through the heart by way of the anterior abdominal vein caused complete inhibition of the heart, was found to be without effect after treatment with the extract (figs. 4, *b* and 4, *c*). The extract caused at first considerable depression of the myocardium but spontaneous beats continued and a later effect was in some instances increased amplitude of contraction with little change in the rate. The response of the heart to excitation of the vagus did not return till some time after the injection of the extract. If however the extract was washed away by perfusing the heart with a quantity of modified Ringer-Locke's solution, the heart as a rule responded to vagus stimulation after a period of from fifteen to thirty minutes' washing. When the stimulation of the vagus with the induced current from the secondary coil of a Harvard inductorium set at zero with a 6-volt current running in the primary failed to produce inhibition, injection of pilocarpine into the heart caused partial inhibition marked by a decrease in the rate of contraction. The injection of 30 cc. of heart extract, made by boiling the macerated tissue with distilled water and filtering and concentrating the filtrate till it was practically isotonic with blood serum, into the left jugular vein of a 12 kilo female dog under ether anesthesia made complete vagus inhibition temporarily impossible to obtain (fig. 4, *e*). Depression of the vagus in the intact dog by splenic extract was not so obtained.

The stimulating action of splenic extract upon uterine muscle was manifested after the tissue had been atropinized (figs. 2, *c* and 3, *b*). Adrenalin caused complete inhibition of the atropinized gravid uterus of the rat, but this was antagonized by splenic extract and further treatment with adrenalin was without effect (fig. 3, *b*). It has been found that the water-alcohol soluble fraction of the splenic extract remains active as regards its ability to antagonize the inhibitory action of adrenalin on certain uteri and to depress the cardiac vagus of the terrapin after it has been freed from certain constituents by treatment with basic lead acetate and silver nitrate in alkaline medium. Also the lead and silver precipitates, after removal of the lead and silver respectively, dissolved in Ringer-Locke's solution failed to produce any such effect.

An acid-alcohol extract of the water-bath dried lipoid-free water-soluble fraction of ox spleen, treated with anhydrous ether, gave a brownish-red precipitate. This was freed from ether and dissolved in Ringer-Locke's fluid. It was found to have no stimulating action on the uterus of the rat and it did not antagonize adrenalin. A slight stimulatory effect was produced upon the isolated duodenum of the rat by this extract. Crawford (7) found that the active principle of the pituitary gland was removed from acid-alcohol extracts by anhydrous ether.

DISCUSSION

The fact that extracts of several tissues antagonize the inhibitory action of adrenalin on strips of isolated uterus which are normally inhibited by it, and also depress the cardiac vagus of the terrapin, suggests that the same constituent or constituents are present in each of these tissues. The point of action in each case is undoubtedly in the peripheral inhibitory nervous mechanism of the organ concerned, probably the myoneural junction itself. Nicotine is without effect after the action of adrenalin on the uterus has been abolished and after the vagus has been completely depressed in the heart of the terrapin. Pilocarpine may have an appreciably stimulatory effect on the uterus after the addition of certain extracts and it is still mildly active on the heart after electrical excitation of the vagus fails to inhibit. Atropine causes slight depression of the uterus after heart or splenic extract have acted and it abolishes the slight action of pilocarpine on the heart of the terrapin with vagus depressed.

As the stimulatory action of splenic extract is still manifested on the atropinized uterus of the rabbit and rat, it is probable that the stimulatory effect is due to the direct action upon the smooth muscle fiber. It is also true that some of the stimulatory effect of certain tissue extracts upon strips of smooth muscle may be due to a depression of the local inhibitory mechanism.

The depression of the inhibitory mechanism which is so clear-cut in the case of certain uterine preparations and of the heart of the terrapin is of interest in another way. The inhibitory nerves of the heart are of the parasympathetic type while those to the uterus are mixed, but in those instances where adrenalin inhibits at all times it is to be supposed that the inhibitory fibers are largely of true sympathetic derivation. Here then (granting that it is the same type of principle which is active in each case) is an instance of certain tissue constituents acting selectively upon the inhibitory nervous apparatus of a tissue irrespective of whether it is of the sympathetic or parasympathetic type.

As the intravenous injection of splenic extract augments the pressor effect of a definite amount of adrenalin, it may be that some part of the vasodilator mechanism which is stimulated by adrenalin (8) is depressed by the splenic extract, and hence a greater pressor response ensues when adrenalin is injected than would otherwise be the case.

As such a heterogeneous group of tissues has been found to contain substances which act in a similar manner in antagonizing the inhibitory action of adrenalin upon certain uteri, and to a lesser degree depressing the cardiac vagus of the terrapin, it is possible that all tissues contain these principles in certain amount. It would in any case seem clear that these particular substances should not be regarded as "hormones."

SUMMARY

1. Extracts of various tissues were found to have definite stimulatory effect upon isolated intestinal and uterine musculature.

2. The primary effect of pancreatic extracts is depression in the tonus of the duodenal musculature.

3. The primary effect of extracts of tissue dried at 110°C. to 120°C. on the intestine is inhibition. This is followed immediately by definite stimulation.

4. Extracts of heart, spleen, pancreas, testes, anterior and posterior lobe of the pituitary body and thymus, thyroid and parathyroid glands in addition to stimulating the uterus of the rat, guinea pig, virgin dog and cat, antagonized the inhibitory action of adrenalin on these organs.

5. Extracts of heart and spleen caused the complete abolition of the response of the cardiac vagus to electrical stimulation.

6. The action of pilocarpine on the heart of the terrapin was partially but not completely antagonized by extracts of heart and spleen.

7. The substance or substances which antagonize the inhibitory action of adrenalin on the uterus and depress the cardiac vagus are soluble in alcohol and resist boiling.

8. Intravenous injection of extract of spleen augments the pressor response to adrenalin.

9. Intravenous injection of extract of heart raises the threshold for vagus inhibition by electrical stimulation of the peripheral end of the cut vagus.

10. It is suggested that substances similar to those found in extracts of various tissues may be present in greater or less amount in all tissues.

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RECIPROCAL REACTIONS IN THE CARDIO-VASCULAR SYSTEM

ETHEL W. WICKWIRE

From the Department of Physiology, Columbia University, New York

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INTRODUCTION

Sherrington (1) in his *Integrative Action of the Nervous System*, has enunciated the doctrine of reciprocal nervous relations existing in the living organism. Isolated facts pertaining to any biological mechanism or to any of its parts are valuable and useful if we can weave from them the whole fabric of truth. But we cannot escape the fact that functional systems, even in lower forms, are closely interrelated and interdependent. As Burdon-Sanderson has expressed it, the processes in the organism go on in the interest of the organism. In man the most complex of willed movements are possible because of the reciprocal innervation of many parts and systems, together with a central seat of integration for his sensory and motor mechanisms. Intake of food, digestion, metabolism and elimination, important as they are, cannot avail much if the blood supply to a part is interfered with by mechanical means, by the blocking out of nervous impulses experimentally, or by accident or disease.

One might expect to find a reciprocal functional relationship between the constituent parts of the cardio-vascular system. This research was undertaken with the object of arriving at a clearer understanding of the reciprocal relationships, whose existence one might be led to suspect, between the cardiac and the vascular nervous mechanisms, through experimental procedures which involved changes in the rate of the heart beat and the magnitude of arterial blood pressure.

From a simple functioning mechanism possibly, in some forms or at some stage of development, independent of nervous connections, the heart has been gradually developed phylogenetically into a complex differentiated organ. Throughout its history its activity has been dependent upon and modified by its increasingly complex physico-chemical environment. At first apparently devoid of nervous relations, later it appeared with nerve fibers connecting it with a more or less simple central nervous system, and it became subject to nervous control. As the central nervous system became more and more complex and highly organized in some of its levels, but less highly organized in others, the nervous connections of the heart multiplied the number of impulses modifying the inherent quality of rhythmicity possessed by the cardiac muscle tissue.

In the course of this development the automatism, originally common to all cardiac muscle, gradually disappeared from some parts, notably the apex. In the higher mammals, therefore, we find a heart with its automatic rhythmicity more and more under the influence of an increasingly complex physico-chemical environment and dependent for its normal activity upon nerve impulses received from the central nervous system.

In the normal animal the rate of the heart beat is closely connected with the blood pressure. If there is a large vasoconstriction, as in cold weather, the accompanying rise of blood pressure is associated with a slowing of the heart beat. Conversely, a vascular relaxation and lower blood pressure are accompanied by acceleration of the heart beat. From observation of these and other related facts Marey (2), in 1860, stated that the heart beat varies inversely as the blood pressure. In the slowing of the heart beat the vagus nerves are of course active, although in certain conditions, for example in muscular exercise, the increased blood pressure is not accompanied by a slower heart rate. It is hoped that the results of experiments recorded in this paper may give some information regarding the nervous mechanism for the adjustment of heart rate to changing blood pressure.

METHOD

The experiments were done on cats. The routine experimental procedure was ether anesthesia, tracheotomy and connection with a mercury manometer by means of a cannula placed in the left carotid artery. Blood-pressure and time tracings were made on a Hürthle kymograph and the heart rate was computed in beats per minute. The stage of anesthesia was determined principally by the presence or absence of the corneal reflex, light anesthesia, however, being always sufficient to abolish any sense of pain. In seeking some way of bringing about changes in blood pressure that could be readily measured without producing any permanent lesions in the blood vessels and with these changes in pressure the consequent or accompanying changes in heart rate, it was found that digital compression of the abdominal aorta was a convenient method. Compression of the abdominal aorta was made just below the diaphragm and yet high enough to avoid any effect by pressure upon the splanchnic nerves. This was accomplished by placing the fingers under the vertebral column, the animal being in the back-downward position on the board, and sliding the thumb in just below the last pair of ribs and dorsal to the viscera until the groove on the ventral surface of the vertebral column is felt. The abdominal aorta lies in this groove, and can be compressed by pressure with the thumb. An alternative method is to slide the second and third fingers in from the side and compress the aorta by pressure with their tips. In order to be sure that no part of the effect was due to pressure on the splanchnic nerves, control experiments were made with a ligature placed around the thoracic aorta by means of a large aneurysm needle passed between the twelfth and thirteenth pairs of ribs. Results of compression of the aorta by means of this ligature coincided with those by means of digital compression. Compression of the descending aorta increases the volume of blood in the anterior portion of the cardio-vascular system and thus raises blood pressure and also increases pressure and velocity of blood flow in the coronary circuit and in the bulbar nerve centers.

Some of the experiments were made with the extrinsic cardiac nervous mechanism intact; others with this mechanism interfered with by certain experimental lesions. These lesions consisted in severing the vagi, removing the stellate ganglia, thus abolishing the influence of the accelerator mechanism, dividing the dorsal roots of the spinal nerves, and dividing both dorsal and ventral roots of the thoracic nerves. Some experiments were also made on the relation of heart rate and blood pressure to hemorrhage. Details of the various types of experiments are given under the respective headings.

RESPONSE OF HEART RATE AND BLOOD PRESSURE TO COMPRESSION OF THE ABDOMINAL AORTA

1. With cardio-vascular nervous mechanism intact. It was desired first to determine the nature of the response of heart rate to changing blood pressures with the cardio-vascular nervous mechanism intact. This was done by compressing the abdominal aorta when the animal was under different stages of ether anesthesia, and also when there had been interference with the blood supply to the bulbar nerve centers. The data are presented in table 1.

By reference to table 1 it is seen that under light anesthesia compression of the abdominal aorta causes the carotid blood pressure to rise immediately; during the continuance of the compression there is a gradual but marked fall in carotid pressure until the aorta is released. Then the pressure falls immediately to a point below its initial level. Subsequently the pressure continues to fall, but soon rises to the level before compression. The high carotid pressure produced by compression of the aorta is accompanied by a marked decrease in heart rate. When the aorta is released and pressure falls the heart rate increases, but does not immediately reach the initial rate. From the averages of fifty compressions upon thirty-five different animals under light anesthesia, there was thus a compensatory response of heart rate to changing blood pressures and Marey's law of inverse ratio of heart rate and blood pressure applies.

TABLE 1

Response of heart rate and blood pressure to compression of the abdominal aorta, with cardio-vascular nervous mechanism intact. Heart rate in beats per minute; blood pressure in mm. Hg, carotid artery

CONDITION	NO. OF EXPERIMENTS	BEFORE COMPRESSION		DURING COMPRESSION		AFTER COMPRESSION	
		Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
Light anesthesia	50	206	124	178	197–166	193	110–76–108
Deep anesthesia	25	197	101	192	164–155	191	102–82–87
Restricted blood supply	23	161	56	158	103–97–100	147	55–46–43
Reversed reaction	7	149	107	164	145–149–140	182	103–84–101

Under anesthesia deep enough to abolish the corneal reflex the initial blood pressure and heart rate are lower than under light anesthesia. Compression of the aorta does not raise the carotid pressure as high as under light anesthesia and there is not the marked fall in carotid pressure during continuance of the compression. When the aorta is released carotid pressure falls immediately to about the initial level. Subsequently it falls more gradually than under light anesthesia, then rises very slowly. The high carotid pressure produced by compressing the aorta is usually accompanied by a very slight decrease in heart rate, but this decrease is wanting in very deep anesthesia. When the aorta is released and pressure falls the rate remains about the same or decreases slightly. From averages of twenty-five compressions on animals under deep anesthesia, it is thus seen that there is usually a slight compensatory response of heart rate to blood pressure when the pressure is high in the medulla oblongata; when the pressure is low, as upon release of the aorta, this compensatory mechanism is seriously interfered with and the return to the initial rate and pressure is either permanently lost or very much delayed.

When the blood supply to the medulla oblongata is restricted, as in hemorrhage or in some instances of partial ligation of the head arteries, blood pressure falls and heart rate decreases. Now when the abdominal aorta is compressed the carotid blood pressure rises immediately, as in light anesthesia, but not to the same extent. During the continuance of the compression, blood pressure at first falls and then rises slightly. When the aorta is released the carotid pressure immediately falls to about the initial level, and then continues gradually downward. When carotid pressure is high during compression of the aorta, the heart rate decreases somewhat, but when the aorta is released and carotid pressure falls the heart rate decreases with the falling pressure. If the anemia of the medulla be severe or long continued, the return to the initial heart rate and blood pressure is lost.

Under certain conditions there appears what may be termed a "reversed reaction." This is sometimes observed if the abdominal aorta is compressed: *a*, during the early period of recovery following excessively deep anesthesia; *b*, during recovery from pronounced asphyxia; *c*, shortly after the reëstablishment of the blood supply to the medulla oblongata after hemorrhage; and *d*, during restricted blood supply from ligation of the head arteries. Instead of a compensatory slowing of heart rate in response to the increased blood pressure, the rate increases during the period of high pressure and continues to increase even after release of the aorta and consequent fall of pressure.

After observing the occurrence of this so-called "reversed reaction" many times in the earlier experiments of this research and noting that it appeared under the conditions above mentioned, it was decided to try to reproduce this type of response under controlled conditions. By referring to reactions 15 and 16 in the protocol of December 23, 1919, (p. 360) it will be seen that this duplication was possible. This reaction follows the recovery of the bulbar centers from anemia produced

Protocol of experiment of December 23, 1919. Ether; tracheotomy. Blood pressure cannula in left carotid artery. Ligature around abdominal aorta between twelfth and thirteenth pairs of ribs. Stage of anesthesia denoted by presence or absence of corneal reflex (c.r.+ or c.r.−). Experiment terminated by the hemorrhages. Sequence of events indicated in "Reaction No." columns.

REACTION NUMBER		RATE	PRESSURE	REACTION NUMBER	BEFORE COMPRESSION			DURING COMPRESSION				AFTER COMPRESSION		
					Heart rate	Blood pressure	Anesthesia	Heart rate	Blood pressure	Anesthesia	Time	Heart rate	Blood pressure	
											seconds			
1	*11:35* After tracheotomy	186	126											
				2	186	126	c.r.+	160	200–146	c.r.+	19	168	92–84–118	
	Lighter anesthesia than above			3	176	112	c.r.+	161	188–146	c.r.+	19	188	98–72–118	
4	Before	186	126											
5	*11:52* After placing ligature around abdominal aorta	156	108											
				6	156	108	c.r.+	133	162–126	c.r.+	19	160	100–82–100	
				7	168	106	c.r.+	151	162–130	c.r.+	19	162	102–80–102	
	Rt. carotid compressed. Light anesthesia			8	162	112	c.r.+	150	174–146	c.r.+	20	159	102–86–104	
	Rt. carotid compressed. Deeper anesthesia			9	*155*	*100*	c.r.+	*157*	*146–140*	c.r.−	*27*	*154*	*104–82–100*	
10	Carotid released—pressure falls; respiration very shallow													
	Artificial respiration			11	212	80	c.r.−	192	148–134	c.r.−	15	189	100–80–90	
	12:01			12	198	106	c.r.−	181	160–186–164	c.r.return	38	200	104–64–94	
	Panting respiration (artificial respiration discontinued)			13	213	98	c.r.+	175	174–140	c.r.++	26	208	94–68–104	Cat restless
	Clamped right carotid and right vertebral			14	216	110	c.r.+	191	204–184	Cat restless	22	212	100–74–124	
					Released R. carotid and R. vertebral.		c.r.+	198	184–144–154		26			
	More ether; deeper anesthesia than 14 Reversed reaction			15	144	106	c.r.++	163	154–132–140	c.r.+	25	189	104–96–100	

	Partial asphyxiation between 15 and 16 Reversed reaction	16	174	96	c.r.+	177	136–144–126	c.r.+	20	192	96–78–88	
	12:14	17	195	108	c.r.+	175	176–144	c.r.++	25	180	100–84–102	
	12:30 Lighter anesthesia (ether out just before 18)	18	186	104	c.r.+	168	184–144–148	c.r.+	41	175	108–82–98	
		19	192	108	c.r.++	167 160 163	184–152 152–158	c.r.+	24 24	165	108–74–94	
20	*12:40* Before (ether intermitted) 186 c.r.+ 120											
21	After hemorrhage of 35 cc. 168 c.r.+ 52											
		22	168	52	c.r.++	160	92–118–124	c.r.+	37	156	84–48–54	c.r.+
	12:44	23	165	60	c.r.+	155	136–124–144	c.r.++	57	135	84–46–56	
	12:49 Ether out; very light anesthesia	24	136	64	c.r.+	123	128–110–120	c.r.++	40	110	84–54–60	
	1:10 Recovery from deep anesthesia	25	192	62	c.r.–	160	138–98–104	c.r.+	53	170	74–40–60	
	1:15	26	170	68	c.r.++	168	142–120–112	c.r.+	50	148	56–36–60	
	1:25 After artificial respiration compression brings back normal respiration	27	189	56	c.r.–	171	84–100–84	c.r.–	46	158	50–32–50	
28	A further hemorrhage of 13 cc. (total of 48 cc.) produces respiratory gasps, a great fall of blood pressure, great slowing of heart and death at *1:54*											

by ligating the head arteries even when the anemia has been severe enough to necessitate artificial respiration. The first of these compressions (reaction 15) was made when the heart rate and blood pressure were falling; the second (reaction 16) shortly after and at a higher level of heart rate and blood pressure brought about by the increased pressure and velocity of blood flow in the medulla oblongata by the previous compression of the abdominal aorta.

The most salient points of the facts above mentioned, when the cardio-vascular nervous mechanism is intact, are: *a*, Under light anesthesia there is a compensatory response of heart rate to changing blood pressures. *b*, Under deep anesthesia and also when the blood supply to the medulla is severely restricted there is a slight compensatory response of rate to pressure when pressure is raised, but when pressure falls this response is completely abolished. *c*, There seems to be a certain level to which the functional activity of the bulbar cardio-vascular mechanism may sink before permanent injury is done to these central cells, a level at which subsequent increase of blood pressure and velocity of blood flow will bring back their function, a previously decreasing heart rate being accelerated and a blood pressure being restored equal to or nearly equal to that before the insult to the bulbar mechanisms occurred.

2. *Response of heart rate and blood pressure to compression of the abdominal aorta, with experimental lesions.* The behavior of the cardio-vascular mechanism to changes in blood pressure when experimental lesions have been produced by sectioning some or all of the extrinsic cardiac nerves is shown in tables 2 and 3. In these tables averages are given to show the type of response of heart rate to changes in blood pressure when the mechanism is intact, for comparison with the types of response when various lesions in the mechanism have been produced experimentally.

In those experiments in which section of the extrinsic cardiac nerves was ultimately desired, the routine procedure was ether anesthesia, tracheotomy, exposure of vagi and dissection for exposure of the stellate ganglia by Anderson's method as described in Sherrington's *Mammalian Physiology* (3).

It will be observed that changes in heart rate and blood pressure due to the operative procedures alone are not great. Marey's law of inverse ratio of rate to pressure holds upon compression of the abdominal aorta after dissection for the exposure of both the vagi and the stellate ganglia, but, in the case of the latter, at a slightly lower level of pressure and rate.

The specific differences in the types of response, when lesions have been produced experimentally in the extrinsic cardiac nerves, are here set forth.

a. Division of vagi, accelerators intact. If the abdominal aorta is compressed after bilateral vagotomy (table 2), when the accelerators are intact, the compensatory response of a lower heart rate to the increased blood pressure remains, but the degree of compensation is much less than before division of the nerves. The level of pressure is about the same as, and the rate slightly higher than, before section of the vagi. When the aorta is released and pressure falls, the compensatory response of a higher rate to a falling pressure is lost and a small decrease in rate accompanies the fall of pressure.

TABLE 2

Response of heart rate and blood pressure to compression of the abdominal aorta, after certain experimental lesions. Heart rate in beats per minute; blood pressure in mm. Hg, carotid artery. Averages from experiments of November 12, 14, 19 and 26, 1919

CONDITIONS IN SEQUENCE OF OCCURRENCE	BEFORE COMPRESSION		DURING COMPRESSION		AFTER COMPRESSION	
	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
After tracheotomy...	223	127	183	202–178	214	104–130
After dissection for stellate ganglia....	184	102	166	173–159	171	99–66–81
After bilateral vagotomy	187	99	181	171–168	178	103–75–76
After excision of stellate ganglia....	161	78	156	135–133	149	87–61–68

b. Division of vagi followed by excision of accelerators. If the abdominal aorta is compressed after excision of the stellate ganglia, subsequent to division of the vagi, there remains the compensatory response of a decreasing heart rate to an increased blood pressure. Both rate and pressure are at a lower level than when the stellate ganglia are intact, the level of pressure being comparatively lower than that of rate. When the aorta is released and blood pressure falls the heart rate does not increase but decreases with the falling pressure.

c. Excision of accelerators followed by division of vagi. The deportment under the conditions *a* and *b* may be contrasted with what occurs when the accelerators are removed first (table 3). Here, immediately after removal of the ganglia, the pressure is found to have fallen greatly and the rate to have decreased somewhat. During compression of the abdominal aorta the cardio-vascular mechanism, after the first sudden rise of pressure, compensates, not by the usual gradual fall, but by a gradual rise of pressure which continues during the whole time of compression; compensation of rate to pressure is greatly diminished, this compensation being entirely lost after release of the aorta and the resulting fall of pressure. Bilateral vagotomy itself, after the accelerators are removed, causes a further small fall in blood pressure but an increase in heart rate. Compression of the abdominal aorta now brings the blood pressure higher, with the same inability on the part of the cardio-vascular mechanism to bring about a fall of pressure during the maintenance of compression. Instead, the blood pressure continues to rise and, when the aorta is released, falls more gradually than before. The compensation of rate to pressure is almost lost during compression and entirely lost when the pressure falls after release.

TABLE 3

Response of heart rate and blood pressure to compression of the abdominal aorta, after certain experimental lesions. Heart rate in beats per minute; blood pressure in mm. Hg, carotid artery. Averages from experiments of October 24, 28 and 29, 1919

CONDITIONS IN SEQUENCE OF OCCURRENCE	BEFORE COMPRESSION		DURING COMPRESSION		AFTER COMPRESSION	
	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
After tracheotomy...	238	155	171	215–184	212	135–112–137
After dissection for stellate ganglia....	198	115	182	181–156	190	105–75–106
After excision of stellate ganglia........	167	63	164	81–91	162	67–53–49
After bilateral vagotomy...............	178	52	177	79-101	170	79–61–49

It is thus seen that when all the extrinsic cardiac nerves are severed either the heart rate falls somewhat and blood pressure considerably, or there may be a greater fall in heart rate and only a moderate fall in blood pressure. The final result seems to depend on whether the vagi or the accelerators are sectioned first. After division of the vagi, when the accelerators are intact, the heart rate usually increases somewhat though the blood pressure may remain the same or may fall slightly.

While there is not sufficient increase in rate to influence pressure, the maintenance of pressure seems in some way to be connected with the accelerators (see Hunt, 4), as is shown by what happens when the stellate ganglia are subsequently removed, for then both the rate and pressure fall.

Removal of the accelerators alone results in a moderate decrease in heart rate and a great fall in blood pressure. Now when the vagi are divided subsequent to excision of the stellate ganglia and the impulses inhibiting the heart rate are removed, the rate increases somewhat though it does not reach the rate it maintained before the accelerators were removed, while the pressure continues to fall. After an interval the latter sometimes rises, which rise may possibly be due to the removal of the "tonic" effect of the cardiac nerves over the heart musculature (5), (6), (7), and a greater volume of blood entering the heart.

The following summary will serve to make more clear the above statements:

Averages from experiments of November 12, 14 and 26, 1919

	Rate	*Pressure*
Before vagi are sectioned	183	98
After vagi are divided	188	96–98
After stellate ganglia are removed	161	80
Percentage fall when vagi are divided first	12	19.2

Averages from experiments of October 24, 28 and 29, 1919

	Rate	*Pressure*
Before stellate ganglia are removed	195	116
After stellates are removed	167	63
After vagi are divided	178	51
Percentage fall when stellate ganglia are removed first	8.7	55

We might add that when the extrinsic cardiac nerves are divided the type of response to compression of the abdominal aorta also seems to depend on whether the vagi or accelerators are divided first, as we have seen above (*a*, *b* and *c*).

With all the extrinsic cardiac nerves cut the heart rate is not so susceptible to the effects of low blood pressure as when the nerves are intact. This is shown by observations made after the injection of sodium nitrite and after hemorrhage. In both of these cases the heart rate is relatively high even when blood pressure is approaching the base line. This is to be contrasted with the gradual simultaneous fading-out of rate and pressure when the nerves are intact.

In this research it has been observed also that the left ventricle is not firmly contracted after section of the cardiac nerves. This is in accordance with previous observations of other structures. It has been found by Bierfreund (cited by Pike, 8) that the onset of post-mortem rigidity is delayed by the hemisection of the spinal cord. This occurs on the hemisected side. Sherrington also found later that the onset of rigor mortis in the hamstring muscles is delayed by section of the appropriate dorsal spinal nerve roots of the same side.

TABLE 4

Response of heart rate and blood pressure to compression of the abdominal aorta, after certain experimental lesions. Heart rate in beats per minute; blood pressure in mm. Hg, carotid artery. Averages from experiments of June 6, 11, 16 and 17, 1919

CONDITIONS IN SEQUENCE OF OCCURRENCE	BEFORE COMPRESSION		DURING COMPRESSION		AFTER COMPRESSION	
	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
After tracheotomy and laminectomy	240	108				
After section of dorsal spinal roots	230	85	196	152–142	200	72–61
After bilateral vagotomy	222	58	214	121–114	201	59–52
After removal of stellate ganglia	187	34	183	71–82–81	165	35–26
After section of dorsal roots						
Light anesthesia	223	78	189	155–134	201	88–64–61
Deep anesthesia	191	58	191	112–101	186	55–47

d. Section of dorsal roots of spinal nerves. The data obtained on the response of the cardio-vascular mechanism to changes in blood pressure after section of the dorsal spinal roots in a region as extensive as cervical 6–7 to lumbar 1–2 are presented in table 4.

Section of the dorsal spinal roots in this region does not abolish the compensation of heart rate to high and low blood pressures. The law of inverse ratio of rate to pressure still holds true and to practically the same extent during compression of the aorta and the resulting increased pressure. This is shown also by the following brief summary from the data of tables 1 and 4.

During compression of the abdominal aorta and high blood pressure.
After tracheotomy, decrease in heart rate = 13.6 per cent.
After laminectomy, decrease in heart rate = 15.8 per cent.
After section of dorsal roots, decrease in heart rate = 14.7 per cent.

When the aorta is released and pressure falls, the degree of increased heart rate to the falling pressure is less after section of the dorsal roots than before:

After release of the abdominal aorta and falling blood pressure.
Before section of dorsal roots, increase in heart rate = 7.7 per cent.
After section of dorsal roots, increase in heart rate = 2.0 per cent.

The similarity of response after section of the cardiac nerves before and after section of the dorsal roots is to be noted. Bilateral vagotomy after section of the dorsal roots produces a somewhat lower level of pressure than it does when these roots are intact. Removal of the stellates, subsequent to bilateral vagotomy after section of the dorsal roots, results in a much lower level of rate and pressure than occurs when these cardiac nerves are divided before the dorsal roots are cut.

Previously it has been noted that after section of the extrinsic cardiac nerves compensation of rate to pressure holds to some extent during high pressure, as during compression of the abdominal aorta, but is lost after release of the aorta and a falling pressure. This is also true when the extrinsic cardiac nerves are divided after section of the dorsal roots, although the fall in rate after release of the aorta is greater than when the dorsal roots are intact. Sherrington (9) notes the general maintenance of arterial pressure after section of the dorsal spinal roots in the thoracic region.

There seems to be a greater sensitivity of the cardio-vascular mechanism to deep anesthesia after the dorsal roots are sectioned than before. This is shown not only by the complete lack of compensation of rate to pressure when the abdominal aorta is compressed, but by both rate and pressure falling to a much lower level than when the roots are intact.

e. Section of dorsal and ventral roots of thoracic spinal nerves followed by stimulation of sciatic nerve. The usual response of an increased heart rate and blood pressure to stimulation of the sciatic nerve is shown in figure 1 and table 5. Here it is also seen that this familiar response to sciatic stimulation seems to be abolished after section of the spinal nerve roots, thoracic 1–5, independently of whether weak or strong stimuli are used.

It is impossible to say, on the basis of the number of experiments done, whether the above lack of response to sciatic stimulation is a necessary effect of section of these roots, or whether it is due to some error of technique which has not yet been detected. Section of these roots, which lie in the region of the emergence of the cardiac accelerators does not, however, abolish the compensatory ratio of rate to pressure upon compression of the abdominal aorta but does alter the type of response of the cardio-vascular mechanism to changes in pressure from that seen when the accelerators alone are removed. Instead of a steady climb of pressure upward, when the accelerators only are removed, after root section pressure first rises and then steadily falls. And there remains a slight compensation of an increased rate to the falling pressure when the aorta is released, which is totally abolished when the stellates only are excised. An increased rate follows bilateral vagotomy after section of these spinal roots as it does when the vagi are sectioned after removal of the stellates. But when the aorta is compressed, after root section and bilateral vagotomy, the pressure rises steadily and much higher than when the cardiac nerves alone are cut, and the heart rate falls with the falling pressure after release of the aorta.

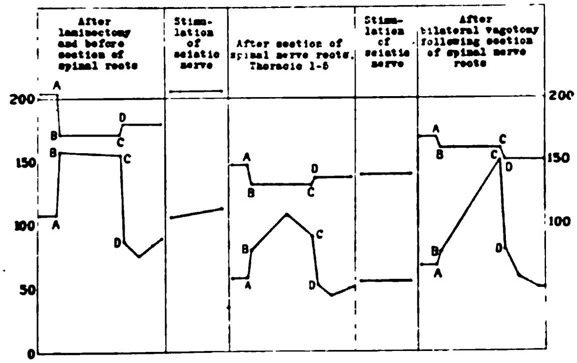

Fig. 1. Response of heart rate and blood pressure to stimulation of the sciatic nerve and to compression of the abdominal aorta, after certain experimental lesions. Lower curves represent the pressure in the carotid artery; figures at the left indicate actual pressures in mm. Hg. Upper curves represent the rate of the heart; figures at the right indicate rates per minute. *A* = point of compression of abdominal aorta; *C* = point of release of aorta.

Further investigation of the results of section of these spinal roots in combination with lesions in other parts of the cardio-vascular mechanism will probably prove of much interest.

TABLE 5

Response of heart rate and blood pressure to stimulation of the sciatic nerve and to compression of the abdominal aorta, after certain experimental lesions.
Heart rate in beats per minute; blood pressure in mm. Hg, carotid artery

CONDITIONS IN SEQUENCE OF OCCURRENCE	BEFORE COMPRESSION		DURING COMPRESSION		AFTER COMPRESSION	
	Heart rate	Blood pressure	Heart rate	Blood pressure	Heart rate	Blood pressure
After laminectomy	203	107	170	157–155	179	86–75–83
Stimulation of sciatic nerve	205	107–113				
After section of spinal nerves, thoracic 1–5	147	58	131	80–109–91	136	53–45–50
Stimulation of sciatic nerve	No effect					
After lateral vagotomy	169	67	160	82–117–152	150	80–58–50

RESPONSE OF HEART RATE AND BLOOD PRESSURE TO HEMORRHAGE

The rôle played by the accelerators is well shown in one particular group of experiments, for when severe hemorrhage is followed by division of the vagi there is a conspicuous increase in heart rate, though not enough to rescue the slightly falling pressure. *The accelerators undoubtedly respond to the emergency of a falling blood pressure.* The summary below presents the data of three experiments.

		HEART RATE	BLOOD PRESSURE
February 10, 1919.	Before hemorrhage	183	150
	After hemorrhage	156	56
	After bilateral vagotomy	160	60
February 17, 1919.	Before hemorrhage	163	162
	After hemorrhage	156	54
	After bilateral vagotomy	204	56
February 21, 1919.	Before hemorrhage	148	120
	After hemorrhage	125	34
	After bilateral vagotomy	141	22
Averages:	Before hemorrhage	165	144
	After hemorrhage	146	48
	After bilateral vagotomy	168	46

The rôle of the accelerators is further elucidated by an examination of the data obtained when hemorrhage follows excision of the accelerators on one or both sides with or without bilateral vagotomy (table 6).

The above table presents some interesting comparisons. A hemorrhage of sufficient volume to cause a great fall in blood pressure before a break in the heart rate occurs, when the cardio-vascular mechanism is intact (1), causes a somewhat greater fall in pressure and nearly a 50 per cent decrease in rate when the accelerators are excised (2). With all the extrinsic cardiac nerves severed (3), the fall in pressure is nearly as great but with only a slightly lower rate (6.2 per cent decrease) than before hemorrhage occurred. With the accelerators of one side intact (4) the fall in pressure is about the same as when all the cardiac nerves are severed but the decrease in rate is somewhat less (1.8 per cent). In the last two instances it is again seen that the rate is not so susceptible to low pressure when all the extrinsic cardiac nerves are cut.

TABLE 6

Response of heart rate and blood pressure to hemorrhage in connection with certain experimental lesions in extrinsic cardiac nerves. Heart rate in beats per minute; blood pressure in mm. Hg, carotid artery

CONDITION	HEART RATE		BLOOD PRESSURE	
	Before hemorrhage	After hemorrhage	Before hemorrhage	After hemorrhage
1. Experiment April 1, 1919.				
Cardiac nerves intact	180	180	100	24
Percentage change		0		75
2. Experiment December 9, 1919				
Accelerators excised (vagi intact)	158	90	80	16
Percentage change		43		80
3. Experiments October 29 and December 17, 1919				
Cardiac nerves excised (accelerators out first + bilateral vagotomy)	160	150	71	16
Percentage change		6.2		77.4
4. Experiment December 13, 1919				
Excision of left stellate; bilateral vagotomy (right stellate intact)	216	210	126	28
Percentage change		1.8		77.7

Various writers have called attention to the fact that the difference in response to stimulation of the accelerators depends principally on the frequency of rhythm before excitation. It seems also that this difference in response depends upon the level of blood pressure during excitation. This is shown by results obtained when the accelerators are stimulated during periods of a diminishing blood volume produced by a series of hemorrhages. When the pressure is low, but the rate yet high, it is found that there is not as great an increase in rate from stimulation as when the pressure has fallen lower from a greater loss of blood and the rate too begins to decrease. It would thus appear that there is a level of pressure at which the effect upon rate from stimulation of the accelerators is greatest, other factors being equal. It may also be said that the accelerators respond to artificial stimulation when the blood pressure has fallen much below this level of greatest response to excitation.

DISCUSSION

In the beginning of this paper it was stated that this work was undertaken with the object of arriving at an understanding of the reciprocal functional relationships existing between the cardiac and vascular nervous mechanisms. That this relationship exists can hardly be doubted when we remember that both heart and blood vessels have their own particular functionally antagonistic innervations. To the heart go efferent fibers that carry impulses from the central nervous system which increase or diminish its rate, and to the blood vessels go efferent fibers that bring about changes in blood pressure by dilating or contracting their vascular walls. The nervous centers specifically concerned with the regulation of this cardio-vascular mechanism, located in the region of the medulla oblongata, have a wide relation with afferent nerve fibers from all parts of the body, including the heart itself. This makes it possible for the heart and blood vessels to be directly or reflexly influenced in their behavior. Changes in rate and amplitude of the heart beat, differences in volume and tone of the heart musculature, and changes in the peripheral resistance may all be brought about by nervous impulses or by changes in the character of the blood flow through the medulla oblongata. With this in mind and remembering that heart rate is closely related to blood pressure and that conditions which affect one influence the other, we can now consider the significance of the differences shown in the behavior of this mechanism when various parts of its regulatory system have been abolished.

The differences, we may say at the outset, are closely related to conditions in the bulbar mechanism. When it is interfered with by partial or complete anemia, asphyxia or too deep anesthesia, the effect on rate and pressure following section of the cardiac nerves is not as great as when the blood supply to the medulla is normal. And we can say also, from observation of many experiments, that the vasomotor center seems more susceptible to such changing conditions when part of the nervous mechanism of the cardio-vascular system is eliminated. When bulbar conditions are favorable there is a reciprocal response on the part of the vasomotors, and blood pressure gradually rises after its first fall following section of the cardiac nerves. If the accelerator branches from the stellate ganglia have been sectioned this rise of pressure is accompanied by a marked increase in heart rate, which is possibly, even probably, due to accelerator impulses that reach the heart through the cervical ganglia (10), (11). But if, instead of a mere section of the accelerator branches, the stellate ganglia have been removed this rise of pressure is unaccompanied by a change in rate. In many other cases of a steadily falling pressure and presumable failure of the vasomotors, pressure can be raised by manipulation of the lower extremities and abdomen and a redistribution of the blood.

The ultimate heart rate and blood pressure after section of the cardiac nerves seems to depend on whether the accelerators or vagi are divided first. In each case the partially intact mechanism apparently adjusts itself to the altered conditions, but the manner of adjustment differs with the sequence of section. With the accelerators removed and the vagi intact, the rise of pressure during manual compression of the aorta is not as great as when the impulses coming over the vagi are also blocked out. It is as if this inhibitory mechanism were standing guard against a rising tide of pressure that might do violence to the best interests of the organism. When the vagi are divided, with the accelerators intact,.pressure from compression of the aorta rises higher than when the accelerators are excised. The fall in pressure, after release of the aorta, continues longer and is greater than before excision of the accelerators. With the accelerators removed it is as if the cardio-vascular mechanism had lost part of its efficient protection against a falling blood pressure and the resultant harm to the multitude of tissue cells.

These results would lead one to the conclusion that when an increased heart rate occurs in the organism through the mediation of the cardio-vascular nervous system, it is due to a positive activity on the part of an accelerating mechanism and not to some mechanism that inhibits the activity of the inhibitors.

This idea of a mechanism of inhibition of inhibition for the regulation of heart rate and blood pressure in the cardio-vascular system may be said to begin with the discovery in 1845, by the Weber brothers, that stimulation of the vagi inhibits the heart rate. E. H. Weber extended the notion of inhibition to the increased spinal activity after ablation of the brain. Following the Webers came Setschenow, who found that stimulation of the midbrain and bulb prolongs reflex time and also that a frog draws its foot from acidulated water much later if the brain be stimulated. But it was not until after his work, in 1863, that "this notion of the inhibition of activity of one part of the central nervous by the activity of another became a working physiological thesis" (12). Later it was shown by L. N. Simonoff (1881) and by A. Herzen (1884) that inhibition of spinal reflexes was obtained from other foci in the brain and cord itself. This was done by sensory stimulation or by direct stimulation of the foci themselves. "But," as Pike has said, "in all this infatuation with the hypothesis of inhibition, its devotees lost sight of the accompanying positive motor phenomena. Until some reason is advanced for this oversight, the hypothesis of inhibition is open to suspicion of being overworked."

In the light of later work on the general life processes of biological mechanisms, it seems that Setschenow's interpretation took color, as so often happens, from the depressing political and economic conditions of his time. If the degree of activity of the inhibiting center is itself dependent upon inhibition, and if the processes in the organism go on in the interests of the organism, as Burdon-Sanderson phrased it, why is there not then this activity of inhibition of inhibition when the accelerators are removed so that the heart rate and blood pressure may be kept up? The activity of the inhibiting vagi, however, is not stopped until the impulses coming over the vagi have been blocked out by double vagotomy or by atropine, or if it is stopped, the results are not of the same magnitude as when the accelerators are present.

It seems rather difficult to apply an hypothesis of inhibition of inhibition for the regulation of heart rate and blood pressure in the cardio-vascular mechanism in view of results obtained in this series of experiments and the facts just stated.

My results however, do, I believe, provide sufficient evidence to postulate a simpler mode of action of the inhibitor-accelerator nervous mechanism. This nervous mechanism consists of: *a*, An inhibitor mechanism acting positively in the best interests of the organism; i.e., to prevent overwork by a too rapid heart rate and to bring about sufficient periods of rest for metabolic repair of the heart musculature. The exercise of this function of the mechanism is not regularly mediated by means of inhibitory influences acting from without upon the mechanism itself; *b*, an accelerator mechanism acting positively in the best interests of the organism and intimately connected with the maintenance of blood pressure. These two functionally different parts of this mechanism possess different thresholds of sensitivity to the same forms of stimuli and varying degrees of sensitivity to each of the various physico-chemical changes occurring within the organism.

Such an hypothesis is in complete accord with the physiological and pharmacological antagonism of other systems innervated, as is this cardio-vascular system, through the cranio-sacral and thoracico-lumbar divisions of the autonomic nervous system.

One further point comes out with regard to the organization and general deportment of the cardio-vascular nervous mechanism, suggested by Prof. F. H. Pike.

The degree of organization, to use Hughlings Jackson's expression, of the neural portion of the cardio-vascular mechanism is high; the responses are, in general, very specific in character, and marked by the absence of any great variety of combinations. Marey's law expresses a definite and relatively unvarying relationship existing between two very definite conditions. The result may be interpreted as being in the interests of the organism. The organization of the central mechanism is not, however, so rigid as to prevent dissociation of these two reciprocal responses of heart rate and blood pressure when the conditions are such that it is in the interest of the organism for this dissociation to occur. As an illustration of this we may cite the deportment of the cardio-vascular mechanism when the blood supply to the medullary portion of it is reduced. If the central mechanism demands a certain definite set of conditions in the way of volume of blood flow and blood pressure for the maintenance of its function, it is obvious that such conditions can be kept uniform only if the whole mechanism has certain powers of adaptation to changing conditions. The adaptation expressed in the sequence of events described by Marey's law is an adaptation to one set of conditions. If conditions should change in such a way as to demand a *high* systemic blood pressure for the fulfillment of the requirements of the central mechanism in the way of volume of blood flow and blood pressure, a falling heart rate in response to such a high systemic blood pressure would be distinctly *not* in the interest of the organism. Haldane has suggested that the rigidity of the deportment of the respiratory mechanism may, under certain conditions, act contrary to the interest of the organism. On that view, the dissociation of the normal response to high systemic blood pressure is to be regarded as an adaptation to restriction of the volume of blood flow through the medulla. It would appear that in the case of the cardio-vascular mechanism as well as in the case of the respiratory mechanism, it is the conditions in the central mechanism itself which, in part at least, determine its degree and state of activity (13).

This dissociation of the usual reactions of the cardio-vascular mechanism is analogous to the dissociation of responses in vertigo, as pointed out by Wilson and Pike.

To Prof. F. H. Pike I offer grateful acknowledgment.

SUMMARY

1. Marey's law of inverse ratio of rate and pressure holds in light anesthesia (deep enough, however, to insure complete anesthesia).

2. This compensation of rate and pressure is lost or seriously interfered with in deep anesthesia; also, in restricted blood supply to the medulla.

3. What may be termed a "reversed reaction" appears under certain conditions of anemia of the bulbar mechanism. There is dissociation of the usual reactions of the cardio-vascular mechanism.

4. The accelerators undoubtedly respond to the emergency of a falling blood pressure.

5. When the accelerators are excised there is a greater percentage change in rate and pressure both falling lower after hemorrhage than when the accelerators and vagi are cut.

6. These experiments demonstrate the tonic activity of the accelerators.

7. The ultimate heart rate and blood pressure, after section of the extrinsic cardiac nerves, depends on whether the vagi or accelerators are excised first.

8. Section of the dorsal spinal roots, cervical 6–7 to lumbar 1–2, does not abolish the compensatory response of heart rate to high and low blood pressures.

9. When the extrinsic cardiac nerves are divided the musculature of the left ventricle remains more flaccid after death than when the cardiac nerves are intact.

10. There is an inhibitor-accelerator nervous mechanism acting positively for the control of the cardio-vascular system.

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FURTHER OBSERVATIONS ON THE RELATION OF INITIAL LENGTH AND INITIAL TENSION OF AURICULAR FIBER ON MYO- AND CARDIODYNAMICS

ROBERT GESELL

From the Department of Physiology of Washington University Medical School and of the University of California

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INTRODUCTION

In a previous paper (1) the relation of initial length and initial tension of auricular muscle to strength of muscular contraction was pointed out in a qualitative way. The present paper represents a more exact and quantitative study of the same problem.

Blix (2) demonstrated in the case of striated muscle of the frog that tension developed is a linear function of initial length of muscle fiber. Evans and Hill (3), employing the same muscle, find heat and tension developed to increase in direct proportion to the increase in length of muscle through an extension of the original unloaded length amounting to approximately 15 per cent. These changes in length, according to Evans and Hill, approximate those normally occurring in situ.

The auricle of the turtle may undergo far greater changes in length of fiber and consequently is a suitable structure not only for determining whether cardiac muscle follows the same laws as striated muscle but also whether the linear function of tension developed holds for these great changes in length of fiber.

It was demonstrated (1) that an increased auricular volume was accompanied by a striking increase in strength of contraction. Though the results indicated at that time that length of fiber was by far the most important factor influencing the strength of contraction yet some results were obtained suggesting that initial tension might play a minor rôle in determining the force of contraction. Consequently methods were used to determine the relative importance of both initial length and initial tension of the cardiac muscle.

METHODS

It is realized that the parallel fibered muscle offers the best condition for studying the strength of muscular contraction; that the interlacing fibers and the reticular arrangement of the bundles of fibers in the auricles may offer difficulties. These difficulties are largely met by recording the pressures developed within the intact auricle rather than the tension developed by a strip of auricular tissue. The method necessarily introduces factors involved in the mechanics of the hollow sphere and the results therefore have an additional interest in supplementing earlier papers on the relation of cardiac volume to cardiodynamics (1), (5), (6) and (7).

The methods employed are similar to those previously described (1). The auricle suspended in saline solution in a stoppered vessel is connected with a membrane manometer and a reservoir also containing saline solution. A simple maximum, minimum and mean valve devised for these experiments is interposed between the auricle and the reservoir to permit the recording of isometric contractions with progressively changing auricular volume (initial length). The changes in auricular volume are recorded with a piston recorder in connection with the air space above the saline solution in which the auricle is supsended.

In the records shown, the upper are the volume tracings, the lower are the tension tracings. Upstrokes represent respectively an increase in volume and increase in tension; downstrokes, the reverse.

In these experiments we are interested not only in the amount of pressure developed by the entire auricular musculature which is recorded directly with the membrane manometer but in the actual amount of tension supported per unit of muscle as well. To determine this we must take into account the changing auricular surface for the same amount of musculature spreads over a changing surface all of which is supporting the recorded pressure. Assuming the auricle to be spherical (it is of course only roughly so) and knowing its volume, the inner auricular surface can be computed. Multiplying this by the recorded initial and final pressure we get the tension per unit of muscle.

RESULTS

The data of this research were obtained from twenty-two experiments on the auricles of nine turtles (Pseudemis elegans). Tables are published for three of these experiments accompanied in two instances by the records from which the data were obtained. The data are tabulated in the following way (see table 1): Column 1—number of contraction; columns 2, 3 and 4—initial pressure, final pressure and pressure developed or the difference between initial and final pressure—all in millimeters of water as recorded by the manometer (these are the intra-auricular pressures sustained and developed disregarding the changing inner auricular surface); columns 5, 6 and 7 represent auricular volume, inner auricular surface and length of auricular fiber in cubic, square and linear millimeters respectively; columns 8, 9 and 10 the tension sustained and developed taking into account the changing auricular surface. These are given in weight (in cubic millimeters of water) sustained by the total inner auricular surface.

Of this data final tension and tension developed are plotted on the ordinates against length of fiber on the abscissas, in figure 1, *a*, *b*, *c* and *d*. An idea of the changes in initial tension sustained is obtained by noting the vertical distance between the curves of final tension and tension developed. In figures 4, *a*, *b*, *c*, *d* and *e* the curves of initial tension are plotted as well.

THE RELATION OF INITIAL LENGTH OF FIBER TO STRENGTH OF MUSCULAR CONTRACTION

Previous results (1) on the auricle of the turtle pointed to initial length of fiber as the important factor determining the amount of tension developed agreeing with the conclusions of Patterson, Piper and Starling (9) on their work on the mammalian ventricle. My conclusions were reached from records similar to figure 3 of the present paper. It will be noted in this record that the strength of contraction progressively increases as the auricle distends and also that the recorded initial pressure, i.e., the pressure obtaining at the onset of auricular systole, is practically constant. Initial length of fiber was apparently the only variable at work influencing the strength of muscular contraction. Unfortunately I had overlooked the factor of increasing intra-auricular surface accompanying the increase in initial length of auricular fiber. We know that whereas the circumference of a sphere increases directly as the radius, the surface of a sphere increases as the square of the radius. Applying this fact to observations on the auricle as illustrated in figure 3 in which initial intra-auricular pressure remains constant, it is obvious that initial length of fiber is not the only variable. On the contrary, since the same amount of muscle is spreading over an increasing surface all of which sustains a constant pressure, the initial tension of the individual fibers increases as the square of the radius whereas the length of fiber increases only as the first power of the radius. Although Patterson, Piper and Starling (9) try to differentiate between the effects of initial tension and initial length of fiber, it appears that they, too, had overlooked the effect which an increasing surface must have upon the initial tension of the individual fibers.

We might in various ways argue the relative effects of initial tension and of initial length of muscular fiber, yet it is more satisfactory to dissociate these factors by direct experiment. We have noted in some few experiments that as the length of fiber increased, a synchronous decrease in initial intra-auricular pressure occurred. Such results were obtained when the auricle was undergoing oscillations of tone by allowing saline to enter the auricle during the phase of tonic relaxation. Since the intra-auricular pressure may decrease out of proportion to the increasing surface and since tonic relaxation per se is detrimental

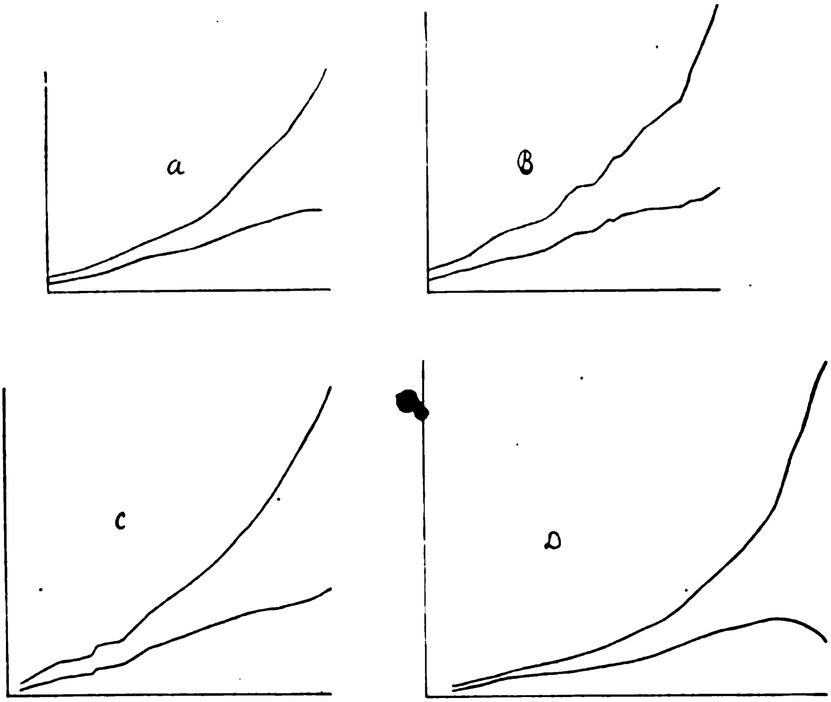

Fig. 1

rather than beneficial to strength of contraction, it appears that the increasing strength of contraction invariably accompanying increasing length of fiber is primarily due to the increasing initial length of fiber.

Having dissociated the effects of initial tension and initial length of fiber under the conditions just described it is of interest to analyze the quantitative data on the relation of strength of contraction to initial length of fiber.

The results of a series of contractions with progressively increasing length of muscle fiber are tabulated and plotted in table 1 and figure 1, *a*. At contraction number 3, the auricular volume is 480 cmm. and at contraction number 70 the volume is 662 cmm. Assuming the auricle to be spherical the length of fiber with number 70 is 60 mm. approximately 196 per cent greater than with number 3. The final tension sustained at number 70 is 1627 per cent greater than the tension sustained at number 3. The tension developed is 930 per cent greater than that developed at number 3.

TABLE 1

(1) CONTRACTION	(2) INITIAL INTRA-AURICULAR PRESSURE	(3) FINAL INTRA-AURICULAR PRESSURE	(4) DIFFERENCE BETWEEN INITIAL AND FINAL AURICULAR PRESSURE	(5) AURICULAR VOLUME	(6) INTRA-AURICULAR SURFACE	(7) LENGTH OF AURICULAR MUSCLE	(8) INITIAL TENSION SUSTAINED	(9) FINAL TENSION SUSTAINED	(10) TENSION DEVELOPED
	mm. H_2O	*mm. H_2O*	*mm. H_2O*	*cmm.*	*sq. mm.*	*mm.*	*cmm. H_2O*	*cmm. H_2O*	*cmm. H_2O*
3	1	2.5	1.5	480	296	30.5	296	740	444
6	1.4	3.8	2.4	835	426	36.6	597	1622	1024
9	1.7	5.0	3.3	1180	508	39.9	863	2540	1676
13	2.4	6.0	3.6	1644	672	46.9	1615	4038	2419
19	3.1	7.1	4.0	2200	818.0	50.6	2535	5807	3272
25	3.7	7.7	4.0	2596	914.0	53.6	3382	1038	3656
31	3.9	8.2	4.3	2931	990.0	55.7	3861	8167	4257
37	5.0	9.0	4.0	3161	1042.0	57.2	5210	9378	4168
43	5.6	9.5	3.9	3335	1078.0	58.2	6036	1024	4204
53	6.2	9.9	3.6	3508	1117.0	59.1	6961	10948	3987
63	6.5	10.2	3.7	3623	1140.0	59.8	7410	11628	4218
70	6.7	10.3	3.6	3662	1148.0	60.0	7691	11824	4182

The curves of final tension and tension developed are plotted in figure 1, *a*. Final tension is represented by a curve with the concavity upwards whereas tension developed is represented by an approximately straight line. Final tension represents two processes, sustenance of initial tension and development of tension. Sustenance of tension presumably is a non-energy consuming process. According to Hill, tension developed represents the energy consuming processes of muscular contraction in which we are particularly interested.

Three similar experiments are represented in figure 1, *b*, *c* and *d*. The tables are omitted. The fluctuations in curves 1, *b* and 1, *c* are due to tonus oscillations and do not concern us here. Ignoring then these oscillations we note again that tension developed is represented by a straight line. Though in some few experiments (see fig. 4) the curve of tension developed is not as straight as in figure 1, *a*, *b*, *c*, *d*, and though the computations for the curves are based on the assumption that the auricle is perfectly spherical, the results on the whole indicate that cardiac muscle behaves to extension as does striated muscle; that in cardiac muscle tension developed is a linear function of initial length of muscle fiber. Of particular interest as concerns the theories of muscular contraction is the fact that this relation holds for such great variations in initial length of muscle fiber, far greater than those occurring in striated muscle.

THE RELATION OF INITIAL TENSION TO STRENGTH OF MUSCULAR CONTRACTION

When an auricle executes a series of isometric contractions with a constant volume the strength of contraction remains constant. If, during such a series of contractions, a tonus oscillation occurs, the strength of contraction varies even though the length of muscle fiber must necessarily remain unchanged. It is found that final tension and tension developed increase with the rise of initial tension synchronous with tonic contraction. These results suggest the beneficial effect of initial tension upon muscular contraction but unfortunately the change in initial tension is a result of a tonus oscillation which in itself may be the factor influencing the clonic contraction.

Another method may be used for dissociating the effects of initial tension and initial length of fiber. If a series of isometric contractions is obtained first with increasing and then with decreasing length of fiber, as in figure 2, it is seen that for any given initial length of fiber the initial tension is higher in the first series of contractions in which the auricular volume is increasing, than in the second series with decreasing volume. Other things being equal, it would follow that if initial tension exerts a beneficial effect on muscular contraction the

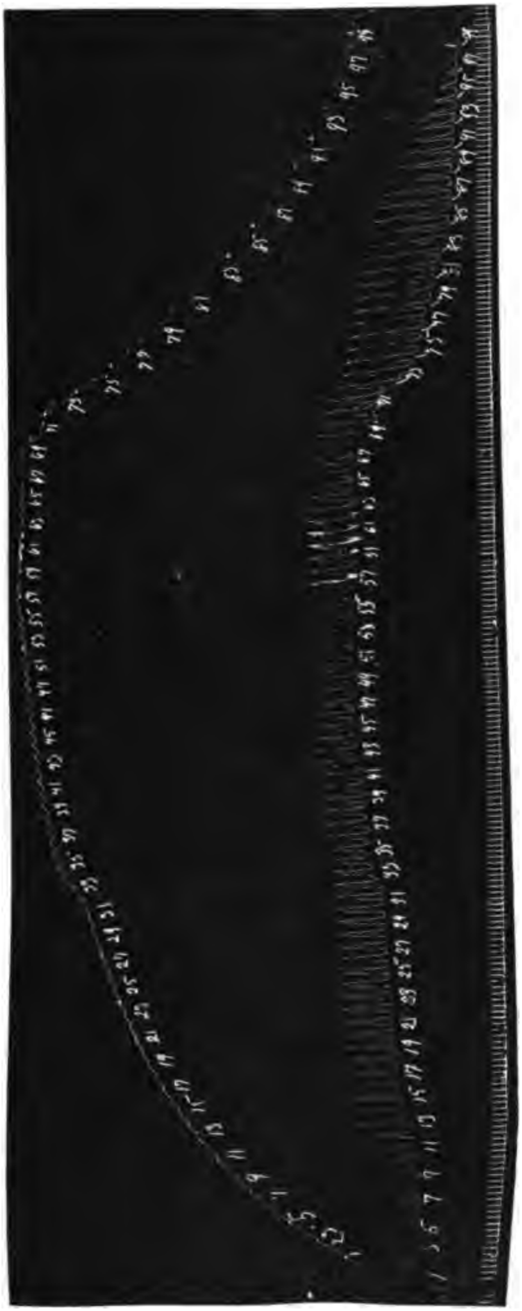

Fig. 2

tension developed per given length of muscle fiber should be greater in the series with increasing length of fiber than in the series with decreasing length of fiber. Before discussing the results, attention should be called to the method employed in obtaining such records and its influence on the interpretation of the results. In the first series with increasing length of fiber, the reservoir is raised to insure an adequate filling pressure. The valve is turned to the maximum position permitting the solution to enter but not leave the auricle. Aside from the diminution in volume permitted by capacity change of the manometer, the contractions are isometric. In the second series with the initial length diminishing, the reservoir is lowered to zero pressure and the valve turned to mean position allowing a small but continuous leakage from the heart back to the reservoir. The resistance in the valve is high enough to permit the development of large pressure changes during contraction so that the contractions approach isometricity but not as closely as the contractions associated with increasing length of fiber. In consequence, a recorded tension in the second series indicates a stronger contraction than the same recorded tension in the first series.

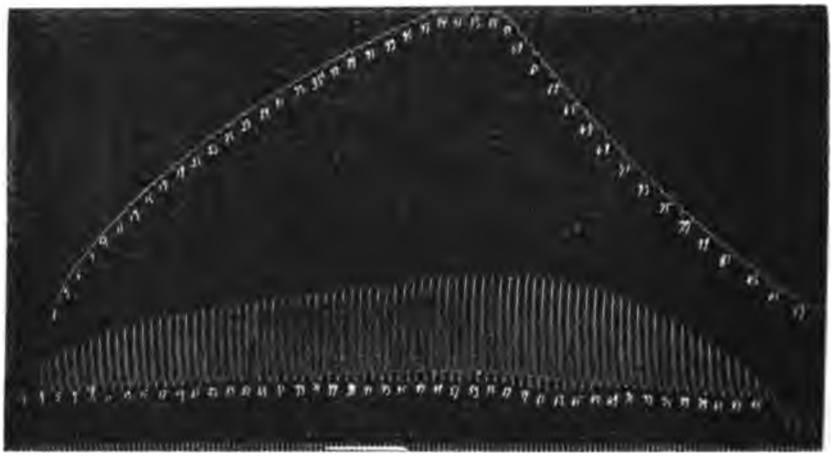

Fig. 3

The data obtained from figure 2 are given in table 2 and plotted in figure 4, *a*, *b* and *c*. Figure 4, *a* represents the curves of final tension, 4, *b*, curves of initial tension and 4, *c*, curves of tension developed. The curves of the first and second series are designated *1* and *2* respectively. Final tensions sustained in the two series are nearly equal. The curve of initial tension sustained is, however, markedly lower in series 2 than in series 1. The curve of tension developed therefore is greater in series 2 than in series 1. Such results might suggest either that initial tension may have a detrimental effect upon muscular contraction or that since the curves of final tension sustained run nearly the same course that muscle at a definite length, other things being equal, is able to sustain a definite final tension—the actual tension developed being dependent upon the level to which the initial tension falls between contractions.

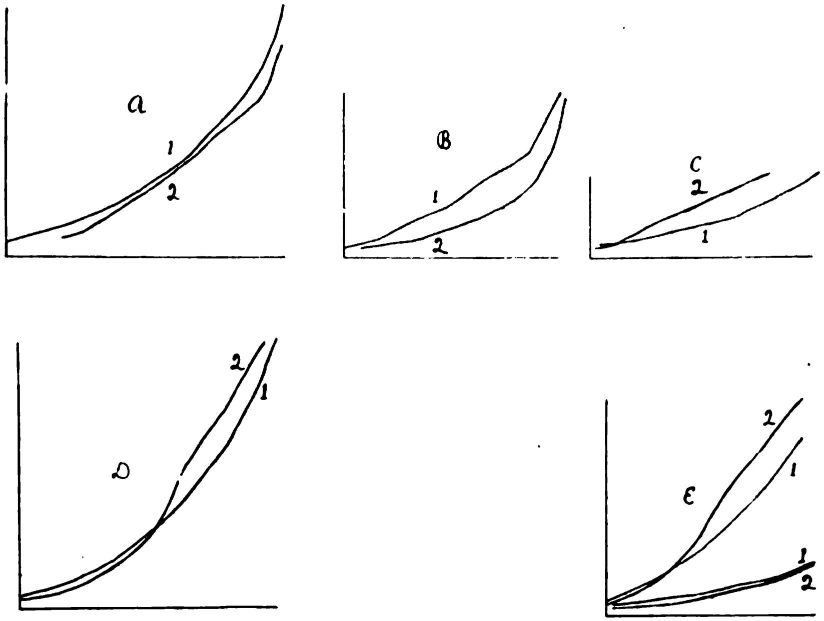

Fig. 4

The experiment represented in figures 3 and 4, *d* and *e*, and table 3 bear on these two suggestions. Here again initial tension is higher in the first series than in the second series. The difference in initial tensions, however, is so slight that a marked effect upon the strength of contraction could hardly be expected. The greater tension developed in the second series is primarily due, in this experiment, to the higher final tension rather than the lower initial tension.

Not knowing of the work of du Bois Reymond (10) and Osborne and Sutherland (11), I sought for more data in the behavior of inflated and

TABLE 2

(1) CONTRACTION	(2) INITIAL INTRA-AURICULAR PRESSURE	(3) FINAL INTRA-AURICULAR PRESSURE	(4) DIFFERENCE BETWEEN INITIAL AND FINAL AURICULAR PRESSURE	(5) AURICULAR VOLUME	(6) INTRA-AURICULAR SURFACE	(7) LENGTH OF AURICULAR MUSCLE	(8) INITIAL TENSION SUSTAINED	(9) FINAL TENSION SUSTAINED	(10) TENSION DEVELOPED
	mm. Hg	mm. Hg	mm. Hg	cmm.	sq. mm.	mm.	cmm. H_2O	cmm. H_2O	cmm. H_2O
1	3.5	4.9	1.4	230	180	23.9	630	882	252
3	3.8	6.4	2.6	508	308	31.1	1170	1986	815
5	4.1	7.0	2.7	728	395	35.2	1606	2765	1058
8	4.6	7.8	3.2	1062	502	39.7	2324	3900	1576
11	5.0	8.5	3.5	1354	592	43.2	2960	5014	2101
14	5.7	9.0	3.2	1584	657	45.4	3777	5913	2135
17	6.5	9.8	3.3	1800	715	47.4	4647	7021	2373
21	6.7	10.5	3.8	2046	779	49.5	5203	8179	2975
24	6.8	10.8	4.0	2220	823	50.8	5579	8888	3308
27	7.0	11.1	4.1	2392	864	52.1	6048	9590	3542
31	7.1	11.6	4.5	2508	893	53.0	6367	10222	3955
35	8.4	12.8	4.4	2815	965	55.0	8106	12352	4246
39	8.6	13.2	4.5	2938	992	55.8	8560	13074	4513
44	9.5	14.2	4.7	3015	1009	56.3	9585	14378	4792
49	9.7	14.7	5.0	3084	1025	56.8	9942	15067	5025
69	9.3	14.7	5.4	3123	1033	56.9	9576	15154	5578
70	7.7	12.3	4.6	3035	1014	56.4	7788	12472	4684
72	7.2	11.7	4.4	2920	988	55.7	7155	11559	4406
73	6.5	10.8	4.3	2838	970	55.2	6305	10446	4141
74	5.0	10.0	5.0	2623	919	53.8	4595	9190	4595
75	4.5	10.0	5.5	2461	881	52.6	3965	8810	4845
76	4.0	9.8	5.8	2296	840	51.4	3360	8232	4872
77	3.7	9.6	5.8	2123	798	50.1	2992	7644	4652
78	3.7	9.5	5.8	2008	770	49.2	2811	7315	4504
80	3.2	8.8	5.6	1750	702	47.0	2267	6177	3910
82	2.9	8.4	5.5	1508	636	44.7	1884	5342	3458
84	2.6	7.8	5.2	1285	572	42.4	1484	4446	2962
86	2.2	7.2	5.0	1108	518	40.4	1129	3729	2600
88	2.0	6.5	4.5	920	457	37.9	958	3004	2066
90	2.0	5.6	3.6	750	399	35.4	798	2254	1456
92	1.8	4.3	2.5	608	347	33.1	625	1492	876
94	1.8	3.8	2.0	480	296	30.5	524	1124	600
96	1.6	2.4	0.8	393	259	28.6	415	642	227

deflated balloons. If one inflates and then deflates a balloon and plots the pressures on the ordinates against the radius or circumference on the abscissas, curves similar to those in figure 5 are obtained. In that the pressures on deflation are lower per given circumference than on inflation they are analogous to the initial pressures in the auricle with increasing and decreasing length of fiber. In this connection it is interesting to note that Langelaan (12) noted a similar hysteresis in the striated muscle of the frog. The question naturally arises, is this hysteresis common to muscle and rubber of significance in the processes of muscular contraction? There is reason to believe (13) that in the case of rubber the change in structure upon which the difference of "initial tension" depends is only temporary (curve *1* can be duplicated after curve *2*) and we might be justified in inferring that in the series of auricular contractions with decreasing length of fiber the structure

TABLE 3

(1) Contraction	(2) Initial intra-auricular pressure	(3) Final intra-auricular pressure	(4) Difference between initial and final auricular pressure	(5) Auricular volume	(6) Intra-auricular surface	(7) Length of auricular muscle	(8) Initial tension sustained	(9) Final tension sustained	(10) Tension developed
	mm. Hg	*mm. Hg*	*mm. Hg*	*cmm.*	*sq. mm.*	*mm.*	*cmm.* H_2O	*cmm.* H_2O	*cmm.* H_2O
6	0.9	2.5	1.6	640	359	33.6	323	897	574
10	1.0	3.3	2.3	980	477	38.7	477	1559	1081
15	1.0	4.1	3.1	1372	596	43.3	596	2443	1847
20	1.1	4.7	3.6	1712	692	46.6	747	3252	2505
25	1.2	5.3	4.1	2028	775	49.3	930	4107	3177
30	1.2	5.7	4.5	2320	847	51.6	1050	4827	3776
35	1.3	6.2	4.9	2588	912	53.5	1185	5700	4524
40	1.4	6.6	5.2	2852	972	55.3	1360	6444	5083
45	1.5	7.2	5.7	3062	1022	56.7	1533	7358	5825
50	1.5	7.6	6.1	3120	1055	57.7	1582	8018	6435
55	1.5	8.0	6.5	3220	1055	57.7	1624	8440	6815
58	1.4	7.8	6.4	3020	1011	56.3	1415	7906	6490
60	1.3	7.7	6.4	2820	966	55.1	1255	7486	6230
65	1.1	7.0	5.9	2260	833	51.1	916	5831	4914
70	1.0	6.2	5.2	1740	699	46.9	699	4333	3634
75	0.8	4.6	3.7	1460	623	44.2	529	2865	2336
80	0.7	2.0	2.2	780	409	35.9	306	1227	920
82	0.7	2.8	2.0	592	340	32.7	248	952	697
85	0.7	1.3	0.6	340	235	27.2	164	295	141

of the muscular elements upon which the contractile processes depend is likewise different from the structure of the muscle during the decrease in length of fiber.

There is another possible explanation also based upon the previous history of the muscle, in this case chemical rather than physical. The staircase phenomenon, for example, is explained on the basis of liberated metabolites formed during contraction, which collect in the muscle and influence subsequent contractions. Applying this to the auricle—as the muscle contracts with progressively increasing length of fiber the amount of metabolites liberated with each contraction presumably increases. In a series with decreasing length of fiber the reverse would occur. But the difference between the two series may be that in series 1 with increasing length of fiber the muscle, so to speak, never catches up with itself in the supply of these products, for with increasing length of fiber, the demands on the muscle are steadily increasing while in the series with decreasing length of fiber it is always ahead of itself for then the demands on the muscle are steadily diminishing.

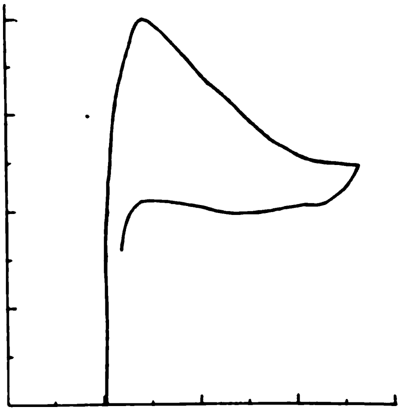

Fig. 5

These explanations of the experiments bearing upon initial tension are admittedly speculative. The results of the experiments, however, have a definite value in pointing to a possible detrimental effect of initial tension upon muscular contraction rather than a beneficial effect. The results enhance the value of the quantitative experiments on the effect of initial length of fiber upon strength of muscular contraction.

A NOTE ON TONUS

A note on tonus. There is an apparent analogy in the behavior of rubber, auricular muscle and the peculiar catch muscle of the bivalve mollusk Pecten described by Parnas (15), Uexkuell (16) and others which may be of significance. The muscle of the Pecten though powerful in that it can support a heavy weight is relatively weak in that its lifting power is small. Recalling the curves of inflation and deflation of the rubber balloon, the analogy is apparent. Since on deflation the balloon contracts and expels air and is therefore lifting, rubber at a given length can sustain a heavier weight than it can lift. How real this analogy is we of course do not know, yet it is very interesting to perform the Pecten experiment upon the balloon in the following manner: If the partially inflated balloon be enveloped and supported by the hand and then compressed to a slightly smaller volume by forcing water into the manometer with which it is connected (which is comparable to passively closing the shells of the Pecten), when the hands are released the water fails by several centimeters to fall to the original level. The rubber again holds more than it can lift.

To be sure the catch mechanism is developed more highly in the catch muscle of the Pecten yet this does not necessarily spoil the analogy for the mechanism is developed to different degrees in rubber just as it is in muscle under varying conditions. From the description of Parnas (15) it appears that in the weakened Pecten an appreciable interval of time must elapse after passive closing of the valves before the muscle will resist stretching. In that respect the analogy between rubber and muscle may be extended, for if an inflated balloon is deflated and promptly reinflated, the curve of reinflation is little higher than the curve of deflation. But if, as Osborne and Sutherland (11) showed, rubber is allowed to "rest" the first curve of inflation may be duplicated. Apparently opportunity must be given for the molecules to rearrange themselves or approximate each other more closely if the force of attraction is to increase. If a balloon just deflated be subjected to a mechanical shock such as slapping sharply in the hand, the first curve of inflation may be promptly approximated. Strong light also tends to restore the properties of recently stretched rubber. It may be that these differences in the properties of rubber under different conditions are due to the formation of molecular aggregates for it has been found (17) that shaking a rubber solution may double its viscosity through the formation of these aggregates. It has also been noted that rubbing of stretched rubber produces microscopic structural changes (13). It may be that the catch mechanism of muscle in its ultimate analysis is not comparable to the phenomenon shown by rubber, yet attention should be called to a possible analogy between a purely chemical or physical phenomenon and a so-called physiological phenomenon.

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THE RÔLE OF THE PANCREAS IN HYPERGLYCEMIA FROM ETHER

ELLISON L. ROSS AND L. H. DAVIS

From the Department of Physiology and Pharmacology, Northwestern University Medical School

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The part played by the pancreas in producing hyperglycemia and glycosuria in diabetics has been the subject of much study. But there has been a tendency to forget the pancreas when dealing with glycosurias and hyperglycemias not directly associated with diabetes (1). We, as well as others, have been guilty of this. In too many instances the attention has been centered only on liver glycogen, nerve supply to the liver, and adrenal secretion. It is well known that the removal of the pancreas will always cause a severe and uncontrollable hyperglycemia and glycosuria. This leaves no doubt that the pancreas exerts an ever-present influence on the mobilization of dextrose in the body. There is no reason to consider that the pancreatic influence may not be subject to the same variation as the influence of any other organs or tissues of the body. Therefore, we have been led to investigate the relation of pancreatic influence on the hyperglycemia resulting from ether anesthesia.

EXPERIMENTAL WORK

A number of healthy dogs were depancreatized. The removal of the organ was accomplished in two operations. At the first, the entire pancreas was removed except the tip of the tail of the gland. This, with its blood supply still intact, was drawn through the abdominal wall and sutured under the skin. At the second operation, about a week later, the remaining portion of the pancreas was removed and the pedicle ligated.

On account of the mortality following anesthesias, we were forced to make observations after the first operation, or on partially pancreatectomized dogs, on one group of animals and observations on completely pancreatectomized dogs of another group.

The group of partially pancreatectomized animals were not glycosuric. They appeared to be in a healthy condition. The effect of ether anesthesia on the blood sugar was determined. About 10 cc. of blood were taken through a hypodermic needle and oxalated. Ether then was administered by inserting the head of the animal into a cylinder into which air that had passed through ether was forced. After fifteen minutes of surgical anesthesia, a second sample of blood was taken in the same manner as before. The dextrose content of the blood was determined by Benedict's method (2). The results expressed in percentage of dextrose in blood are given in table 1.

TABLE 1

Effect of ether on glycemia of partially pancreatectomized animals

ANIMAL	DEXTROSE IN BLOOD		
	Before ether	After 15 minutes ether	Increase
	per cent	*per cent*	*per cent*
1	0.110	0.189	0.079
2	0.100	0.120	0.020
3	0.083	0.111	0.028
4	0.081	0.112	0.031
Average	0.0935	0.1330	0.0395

TABLE 2

Effect of ether on glycemia of completely pancreatectomized animals

ANIMAL	DEXTROSE IN BLOOD		
	Before ether	After 15 minutes ether	Increase
	per cent	*per cent*	*per cent*
5	0.344	0.400	0.056
6	0.344	0.392	0.048
7	0.256	0.322	0.066
8	0.285	0.327	0.042
9	0.287	0.300	0.013
10	0.377	0.400	0.023
Average........	0.3155	0.3568	0.0413

The second group of animals consisted of those which had had a complete pancreatectomy. They all had glycosuria. They were all active, bright and ready to eat and drink. They were given a meat diet exclusively. Two of the animals were treated exactly like those of the preceding group. On others of this group it was thought best to measure the changes in the output of urinary sugar as well as blood sugar. Therefore a catheter was passed through the urethra into the bladder and left in place throughout the test. The bladder was first emptied and was then washed out with 10 cc. of water three times. The animal was kept on the board half an hour and at the end of that time the urine was drawn off and the bladder washed out exactly as before. The urine and bladder washings were combined and analyzed for dextrose according to Benedict's method (3). A sample of blood was taken and ether was given in the usual manner described above. After fifteen minutes of anesthesia a second sample of blood was taken. At the end of half an hour of anesthesia a third blood sample was taken and the urine collected as before. The results expressed in percentage

TABLE 3

Dextrose changes in blood and urine of pancreatectomized animals induced by ether

ANIMAL	DEXTROSE IN BLOOD		DEXTROSE IN URINE OF ½ HOUR BEFORE ETHER	DEXTROSE IN URINE OF ½ HOUR DURING ETHER
	Before ether	After ½ hour ether		
	per cent	*per cent*	*grams*	*grams*
7	0.256	0.327	0.411	0.062
8	0.285	0.340	0.719	0.242
9	0.287	0.303	0.336	0.227
10	0.377	0.410	1.032	0.515

TABLE 4

Dextrose changes in blood and urine of pancreatectomized animals induced by ether

ANIMAL	INCREASE IN DEXTROSE IN BLOOD DUE TO ½ HOUR ETHER	DECREASE IN DEXTROSE IN URINE DUE TO ½ HOUR ETHER	CHANGE IN BODY DEXTROSE DUE TO ½ HOUR ETHER
	grams	*grams*	
7	0.574	0.349	+0.225
8	0.363	0.477	−0.114
9	0.101	0.109	−0.008
10	0.267	0.517	−0.250
First average.......	0.3262	0.3630	−0.0368
Second average.....	0.2436	0.3676	−0.1240

NOTE: The first average is the average of all. Second average omits the values for the first animal.

of dextrose in blood are given in table 2. The results expressed in percentage of dextrose in blood and grams of dextrose in the urines for two half-hours are given in table 3. Deductions from table 3 are given in table 4.

DISCUSSION

In table 1 is expressed the effect of fifteen minutes of ether anesthesia on the blood sugar of animals possessing only a small fraction of pancreas and that in an abnormal location. The animals were undieted. There was the usual individual variation which makes individual changes of much less significance than the average from a group of animals. However, it is worth noting that there was a decided increase in the glycemia of every animal. The average blood sugar before anesthesia was 0.0935 per cent and after anesthesia 0.133 per cent of blood dextrose. The average increase in these animals was 0.0395 per cent. In a previous paper (4) the effect of fifteen minutes of ether anesthesia on the blood sugar of seventeen normal dogs was reported. The average blood content of dextrose before anesthesia was 0.090 per cent and after fifteen minutes of ether anesthesia produced in our usual way the bloods averaged 0.127 per cent. The increase was 0.037 per cent. The close agreement between the effect of ether on normal dogs and dogs with much less than the normal pancreas, leads us to conclude that an influence on carbohydrate metabolism equal to normal was being exerted by these small pieces of pancreas implanted under the skin. Ether disturbed the equilibrium just the same as in normal dogs.

In table 2 is expressed the percentage of blood sugar before and after anesthesia of animals without a pancreas. The high glycemia is striking. It is fully three times the amount normally found. All the animals had severe glycosuria. The average dextrose content of the blood before ether was 0.3155 per cent and after fifteen minutes of ether anesthesia 0.3568 per cent. There was an average increase of 0.0413 per cent. It is notable that the values before and after ether were particularly high and that the increase agrees well with the hyperglycemia of normal animals. If there were no changes in the rate of dextrose excretion by way of the urine, this agreement between the increase of normal and pancreatectomized dogs would be of considerable significance.

In order to determine whether there was any change in the dextrose excretion brought about in these pancreatectomized dogs by ether anesthesia, the dextrose excreted the half-hour before ether and the half-hour during ether anesthesia was determined. From table 3 it is noted that in the half-hour there was a decided increase in the blood sugar percentages and also a decided decrease in the grams of urinary dextrose. In table 4 it is possible to compare the blood increases with the urine decreases. The blood increases expressed in grams were calculated from the percentages of dextrose, the weights of the dogs and Howell's blood and body weight factor (5) of 7.7 per cent.

With the exception of dog 7, there was in every case less increase in blood sugar than there was decrease of urine sugar. The possible cause of this exception was that during the anesthetic dog 7 stopped breathing and nearly died. The asphyxia in this case was severe and capable of causing marked disturbances of the carbohydrate regulatory mechanism. The average which includes all the animals, shows a decrease in dextrose mobilization of 0.0368 gram due to ether anesthesia of the half-hour. The second average which does not include dog 7 shows a reduction in dextrose mobilization of 0.124 gram. There is no doubt that the removal of the pancreas removed at least one of the sites of action of ether in its production of hyperglycemia.

Cannon (6) worked out the mechanism of the mobilization of dextrose by pain, fear and rage. These factors are capable of stimulating the nerve endings in the adrenals, a stimulation which results in the freeing of epinephrin into the blood stream. This epinephrin acts directly on the glycogen of the liver to set free dextrose. Stewart objects to some of these findings (7).

Macleod (8) has presented data which leads him to conclude that sympathetic nerve terminations controlling glycogenic function can be stimulated either by excess of adrenalin in the blood or by nerve impulses alone.

It is a well-known fact that the removal of the pancreas causes a disappearance of glycogen from the liver and the production of an uncontrollable hyperglycemia and glycosuria. Allen (9) concludes that derangement in the internal secretion from the pancreas is the factor most concerned in the etiology of diabetes. The pancreatic internal secretion must have an inhibitory effect on the liberation of glycogen, since the removal leads to a rapid disappearance of glycogen.

Thus we have strong experimental evidence that there are three important influences being exerted on stored glycogen, i.e., adrenalin, sympathetic nerve endings and pancreatic internal secretion. The nerve endings and adrenalin exert an influence to liberate dextrose from glycogen. Pancreatic internal secretion exerts an influence to prevent the liberation of dextrose from glycogen. So there is for glycolysis an inhibitory and an acceleratory mechanism, a mechanism which is common to many of the body functions. If this be true, no discussion or investigation of any condition which has to do with the mobilization of dextrose is complete unless all these factors are considered. There is no more reason for regarding the internal secretion of the pancreas as invariable than for so regarding the internal secretion of the thyroid.

Our results show that ether does not bring about the mobilization of sugar in the animal without a pancreas, as it does in a normal one. Since the pancreas inhibits glycolysis, we conclude that ether reduces the activity of the pancreatic internal secretion of normal dogs, and thus increases dextrose mobilization. It may be objected that the store of glycogen was so depleted after pancreatectomy that dextrose could not be liberated in normal amounts. This objection will not hold because large amounts of dextrose were being freed into the blood and excreted by the urine hourly as was indicated by the blood and urine content stated in the tables.

Keeton and Ross (1) presented data suggesting that the action of ether to cause hyperglycemia was through hepatic nerve influence and not chemical action directly on the liver cells. They did not take into consideration the internal secretion of the pancreas. Because they did not get hyperglycemia with ether in bi-splanchnectomized animals, they concluded that ether could not be working on the liver directly. The most probable cause for this result, under these conditions, was that the cutting of the nerves to the adrenals reduced the epinephrin output more than ether reduced the internal secretion of the pancreas. Since the adrenals encourage glycolysis and the pancreas inhibits it, the result would be an absence of a hyperglycemia, a result which was obtained.

In a previous publication (10) it was found that chloroform hyperglycemia was not reduced by atropin as was the case with ether hyperglycemia. The mechanism of the two must have certain differences. In view of the present conclusions and since we know chloroform seriously injures liver cells, it is possible to say that the results were due to the inability of the injured nerve endings in the liver and the adrenalin to accomplish as much change in glycogen stored in cells whose walls had been injured and whose chemical equilibrium had been altered, as normally. So that possibly chloroform reduces the pancreatic internal secretion exactly the same as ether but in addition injures the liver, which would result in not so great a hyperglycemia as that of ether and also not be subject to influences of such drugs as atropin while ether is so influenced.

Stewart and Rogoff (7) have presented much data contending that ether hyperglycemia is not due to the liberation of excess adrenalin into the blood stream. They have cut the splanchnics on one side and removed the adrenal gland on the other side. The cats were allowed a number of days to recover. Then they were anesthetized with ether. A hyperglycemia resulted. The amount of adrenalin in the blood before and after the anesthesia was far below normal. During the time allowed for recovery there is no doubt that there was a readjustment between the accelerator and inhibitor glycolytic mechanisms, as is commonly seen after a disturbance of one of the mechanisms governing heart rate. So at the end of several days the inhibitor glycolytic mechanism, the internal secretion of the pancreas, had fallen to a lower level more nearly equal to the accelerator glycolytic influence of adrenal and hepatic nerve ending activity. So when ether was given, according to our view the activity of the pancreas was reduced and the accelerator glycolytic mechanism became the more powerful and dextrose was set free into the blood stream in greater abundance.

SUMMARY AND CONCLUSIONS

The hyperglycemia induced by ether was measured on two series of dogs. One group had partial pancreatectomies and the other group had complete pancreatectomies. The effect on the urine dextrose of ether anesthesia was also measured in the case of the pancreatectomized dogs.

The hyperglycemia induced by ether anesthesia of partially pancreatectomized dogs was the same as that of normal dogs.

The hyperglycemia induced by ether anesthesia of completely pancreatectomized dogs was practically the same as normal. Comparing the dextrose output in the urine half an hour before anesthesia and half an hour during anesthesia showed a marked decrease in the elimination of dextrose. Comparing the rate mobilization of dextrose with and without ether showed that it was markedly decreased by the drug.

The results lead us to conclude that the chief action of ether in causing a hyperglycemia in normal animals is to reduce the influence of the internal secretion of the pancreas on glycolysis.

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PHYSIOLOGICAL STUDIES ON PLANARIA

IV. A Further Study of Oxygen Consumption during Starvation

LIBBIE H. HYMAN

From the Hull Zoölogical Laboratory, University of Chicago

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INTRODUCTION

In the first paper of this series (1) it was shown that in Planaria dorotocephala the rate of oxygen consumption rises during starvation. This rise is readily detectable in less than three weeks after the cessation of feeding; and from this time on the oxygen consumption rises continuously with an acceleration for at least eight weeks, when the experiments were concluded. This result agrees with the result previously obtained by the susceptibility method. The susceptibility method consists in observing the time of death of organisms in lethal solutions. It is believed on adequate grounds, which need not be reiterated here since they have been discussed in numerous papers from this laboratory, that such time of death is in a general way a measure or indication of the metabolic rate of organisms. By this method it had been found many years ago by Professor Child that the susceptibility of planarians is increased when they are starved and that it is greater and their survival time in lethal solutions shorter the longer the period of starvation has endured. From this result Child drew the conclusion that the rate of metabolism is increased by starvation. My results on the rate of oxygen consumption confirmed the conclusion reached by Child through the susceptibility method and further supported Child's general conceptions concerning the nature of senescence and rejuvenescence (3).

Meantime, however, Lund and Allen at the University of Minnesota sought to find evidence against Child's viewpoint. Their point of attack concerns the susceptibility method and they have been attempting to show that the susceptibility method is not a reliable measure of metabolic rate. Their arguments reveal a certain amount of misunderstanding concerning the application of the susceptibility method but the matter is perhaps of little consequence now, since the main conclusions drawn by the use of the susceptibility method have been verified by quantitative determinations of oxygen consumption and carbon-dioxide production.

In a paper appearing simultaneously with mine already referred to (1), Allen (2) sought to show that in Planaria agilis and Planaria maculata the rate of oxygen consumption is not increased during starvation within the periods tested by him (nine weeks). In this paper he also made an elaborate comparison between the susceptibility of planarians in various physiological conditions and their rate of oxygen consumption under the same conditions and argued that since the two methods do not (according to him) always yield the same results, the susceptibility method is not a reliable index of the rate of respiratory metabolism.

This comparison made by Allen would probably appear convincing to anyone not conversant with all of the facts. But it has in reality no adequate scientific basis since the comparison is made *between the susceptibility data obtained by us on Planaria dorotocephala and the oxygen consumption data obtained by him on Planaria agilis and Planaria maculata.*[1] Allen at the time of writing did not know anything about the *rate of oxygen consumption* of P. dorotocephala during starvation. How then can he maintain that it does not agree with the findings by the susceptibility method? As a matter of fact there is in this species as I have shown (1) general agreement between the susceptibility results and the rate of oxygen consumption; both show that metabbolism is markedly increased by starvation. On the other hand Allen did not know or attempt to find out anything about the *susceptibility* of P. agilis during starvation. How then can he maintain that in this species there is a discrepancy between the results by the susceptibility method and the oxygen consumption data? My own findings demonstrate that no such discrepancy exists. It is true that the rate of oxygen consumption in P. agilis increases very slowly during starvation and the increase appears only after four to six weeks while in P. dorotocephala it is present before the third week of starvation; but it is also true that in P. agilis the *susceptibility to potassium cyanide shows no or little increase during this period.* This fact completely demolishes Allen's argument. If he had taken the trouble to investigate the susceptibility of P. agilis during starvation, he would have realized that he had no basis for his contentions. As a matter of fact, the conditions in P. agilis, far from disproving the value of the susceptibility method, furnish a remarkable demonstration of its utility; for the differences between the two species could have been discovered by means of the susceptibility method alone.

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[1] Owing to the uncertainty in the identification of the species Planaria maculata, explained later, consideration of this species is left out of the discussion.

Marked differences exist in the physiology of P. dorotocephala and P. agilis; these differences are mostly quantitative and not qualitative. P. agilis respires about two-thirds as fast as P. dorotocephala, it consumes more food and has probably larger food reserves, it loses weight more slowly in starvation, and requires a much longer period to reach the same degree of rejuvenescence. Such differences must receive consideration in experimental work. To assume, as Allen did, that because P. dorotocephala reaches a high degree of rejuvenescence within a few weeks, other species must also, and if they do not the conclusions drawn from P. dorotocephala are erroneous, is an unscientific proceeding. Each species must be thoroughly analyzed and studied as we have studied P. dorotocephala before comparisons can be made.

The question next arises: is it true that in P. agilis and P. maculata, as maintained by Allen, the oxygen consumption does not increase within nine weeks of starvation? I have repeated Allen's experiments upon these species (cf., however, footnote 1) and find that he was mistaken in his conclusions. A critical examination of the data presented by him shows that they are inadequate and unconvincing and furnish an insufficient basis for his contentions, especially in view of the fact that those contentions are counter to a large body of evidence already at hand.

Allen has presented six experiments, three on P. agilis and three on P. maculata. In but one of the six has the oxygen consumption been tested from the beginning to the end of the period of starvation. This experiment is given in his table 7. At the end of this experiment the worms were consuming some 40 per cent more oxygen than during the early part of the experiment. The result, therefore, in the one really adequate experiment performed by Allen is exactly the contrary to his general conclusion. My experiments show that the results given in this table are essentially correct.

I wish to consider each of his experiments in a little more detail. Tables 4, 5 and 6 deal with P. maculata. In table 4 the experiment is begun with worms which had already been starving for "several weeks." We are not told just how long this period was nor do we know anything about the rate of oxygen consumption during this part of the starvation period. The oxygen consumption of these worms was then tested during thirty-four days of starvation, during which it is said by Allen to be "constant." This "constancy" means a variation of from 5.8 to 8.2 cc. of oxygen per gram per day. This variation is much greater than that regarded in other tables as of significance and clearly indicates a lack of experience with the method. Leaving this irregularity out of consideration, it may be said that the data in the table are of no significance since after "several weeks" of starvation a high level of metabolism has already been attained by some species of Planaria and this rate may not increase further. Unless we know the complete history of such worms the data are of no value for the purpose. The reader should note, however, that according to table 5 the average oxygen consumption per gram per day after "several weeks" starvation is *6.9 cc.* Turning now to table 5, we find that in the same species of Planaria between the fifteenth and forty-second days of starvation the average oxygen consumption per gram per day is about *4.5 cc.;* and again in table 6, between the fifth and twenty-ninth days of starvation it is *5.2.* This discrepancy between the results in table 4 on the one hand and those in tables 5 and 6 on the other was not mentioned by Allen; but it is perfectly evident that it can be explained only on the basis that the oxygen consumption is decidedly increased after "several weeks" starvation. We may therefore state that Allen's data on P. maculata are inadequate because in no case was the oxygen consumption determined throughout a period of starvation covering a number of weeks; and that as far as they show anything at all they certainly do not support Allen's conclusion but lead in the opposite direction.

Allen's tables 2, 3 and 7 deal with P. agilis. In table 2 it is shown that the oxygen consumption in this species is less after three weeks than after one day of starvation. This is also the case in all species of Planaria which have been tested. The greater rate after one day of starvation is due to the persistence of the effect of feeding on the digestive tract as explained in a previous paper (1). This table has therefore no direct bearing on the question of the rate of metabolism during starvation, since the comparison must be made with worms in which the increased oxygen consumption due to feeding has been eliminated. In Allen's table 3 data are given which show no difference in the rate of oxygen consumption between worms starved one week and worms starved nine weeks. These data are most certainly incorrect. They are directly contradicted by the results in Allen's table 7, in which after nine weeks' starvation an increase of about 40 per cent in the rate of oxygen consumption has occurred. Perusal of the text referring to table 3 throws some light on the origin of the error, since it is stated that the control worms had been kept in the laboratory only a few days preceding the test. Since the metabolic rate of worms decreases when they are maintained under laboratory conditions it is probable that the control worms in this case had a higher rate of oxygen consumption than they would have had if kept in the laboratory the same length of time as the experimental worms.

As already noted, the experiment recorded in table 7 is the only one presented by Allen in which the metabolism of the worms was studied from the beginning to the end of a starvation period lasting about ten weeks. This experiment is also the most accurate, as Allen states. Unfortunately at the critical point in the experiment, a gap of three weeks is present. Nevertheless the results clearly show that the oxygen consumption of P. agilis is increased by starvation, a rise of about 40 per cent having occurred after nine weeks' starvation. Allen attempts to explain away this result on trivial grounds, but my own experiments on five different lots of P. agilis, each of which was studied throughout a period of at least twelve weeks' starvation, prove that it is essentially correct. The rate of oxygen consumption of P. agilis like that of other species of Planaria is increased by starvation; but a longer period of time is required for the increase to occur.

It is evident that throughout his experiments Allen proceeded on a physiologically incorrect assumption. He supposed that absolute length of time is the important factor in starvation; whereas in fact the primary factor is *the rate at which starvation proceeds.* This rate is determined by a number of factors other than time, such as: the temperature during starvation, the age (size) of the worms at the beginning of the experiment, the metabolic rate of the species in question, the rate at which weight is lost, and the amount of food reserves present in the body. An increase in metabolism as a consequence of starvation cannot be expected to occur until the animal actually begins to use its own tissues for food and the time required for this is variable with different species and under the different circumstances just enumerated.

EXPERIMENTS ON PLANARIA MACULATA

1. Source and care of material. The so-called species Planaria maculata lives in the Chicago region in small still bodies of water. It is found creeping about on the submersed vegetation. The stocks of worms used in these experiments were collected from the lagoon in Jackson Park in the city of Chicago. This lagoon to the east of the Wooded Isle is filled with submersed water weeds, chiefly Elodea, Ceratophyllum, Myriophyllum and Potamogeton. Large quantities of these plants were brought in, packed closely in dish pans, and enough water added to just cover the plants. Within two or three days the plants begin to decay and as the planarians cannot endure such conditions they creep up to the surface layers of plants, and after more time has elapsed will collect at the margins of the pans near the surface. If the surface layers of plants are removed after two or three days, placed in pans with a large quantity of water, and violently agitated, the worms will fall off to the bottom and begin to crawl up the sides of the pan to the top. They can then be readily picked off and placed in pans of clear water. Although the method is rather laborious and time-consuming, it is possible with a little patience to accumulate a considerable stock of such worms. They are most abundant on the vegetation in the autumn.

Stocks so collected were kept in large pans of well water. If they are to be maintained for any length of time, they must be fed. I at first tried to feed them by the same method we were accustomed to use for P. dorotocephala, namely, by placing pieces of liver in the pans. It was at once found that this species will not take food by this method. It was then suggested by Professor Child that this species is so sluggish and insensitive that it does not detect food except at very short distances and that the liver should be ground and strewn over the bottom of the pan. This procedure was tried but was even less successful than the preceding plan, because not only did the worms refuse to feed but many of them died as a consequence of the presence of the ground liver in the pan. While giving this plan a trial, however, the correct method of feeding was accidently discovered. In an attempt to reduce the labor of removing the ground liver from the pans after feeding it was decided to wash the liver, since blood and other materials diffusing from the liver render the water so opaque that the worms can no longer be seen. The liver was then ground and washed and it was at once observed that the worms would feed voraciously on such washed liver. Evidently some materials in the liver (bile?) are injurious to this species. The feeding plan adopted was then the following: the liver was ground in a meat grinder, poured into a strainer, and a stream of water run through it until the water came through clear. The washed liver fragments were then strewn over the bottom of the pan and the worms stirred up and washed down from their usual resting places along the sides of the pan. After three or four hours, the worms, having fed, again accumulate on the sides of the pan, and the liver fragments can be removed with the aid of a suction pipette made of an atomizer bulb and a glass tube of wide bore. The worms and the pan are then washed thoroughly two or three times to remove all fragments of liver. By this method the worms feed readily and grow with astonishing rapidity.

2. Taxonomy of Planaria maculata. Inspection of a stock of worms collected as described above at once shows that two distinct types are present. Neither of these corresponds to the description of the true Planaria maculata of the eastern United States. I am of the opinion that there are at least three distinct species to which the name Planaria maculata has been applied indiscriminately.

One of the types occurring in collections from pond weeds has the following characteristics: the body is short and relatively broad and not at all or but slightly narrowed behind the auricles; the pigmentation varies from chocolate to ashy brown and is decidedly arranged in spots with conspicuous white blotches between; there is never any white stripe down the center of the dorsal side but large specimens show a tendency to develop a dark stripe in this region. This type is designated in this paper as the spotted variety. It is not found sexually mature in nature but sexually mature individuals have developed in our stocks and are at the present writing laying capsules. An investigation of the morphology of the reproductive system in this form will settle the question of its identity or non-identity with Planaria maculata.

The other species present in our stocks has the following appearance: the body is longer and more slender than in the spotted variety and very much narrowed behind the auricles; the pigmentation is more evenly distributed and to the naked eye not at all spotty in arrangement; it is of a very dark brown, almost black color and the white part of the eyes stands out very conspicuously in consequence; there is always a well-marked white stripe down the center of the back. This form is designated in this paper as the striped variety. Although specimens in our stocks have attained a considerable increase in size, no sexually mature individuals have as yet appeared.

Both of these forms are different from the true Planaria maculata which I have observed at Falmouth, Mass. The general proportions of the latter are similar to those of the spotted variety but it is not nearly so spotty in appearance and generally bears a white stripe down the center of the back. Further, it lives in a different kind of habitat, under stones in clear, comparatively quiet water. This species does not live in the Chicago region as the type of habitat necessary for it does not occur here. Finally its behavior in regeneration experiments is quite different from that of our two varieties.

In view of this confusion regarding the taxonomy of this species, it is impossible to know whether investigators working with what is called P. maculata are really using the same species or not. This difficulty arises in connection with Allen's experiments. It is not certain that the species used by Allen is the same as either of the two varieties used by me in the present experiments. From some remarks made by Allen to Professor Child at the 1919 meeting of the American Society of Zoölogists, it seems probable that he was working with the true Planaria maculata. At any rate the results which I have obtained are quite different from his and this difference may be due in part to the use of different species.

3. Method of procedure. From the general stocks lots of worms were selected, each consisting of some two hundred worms of approximately the same length. Two such lots were selected of the striped variety and three of the spotted variety. Each lot was kept in a separate dish and its rate of oxygen consumption determined at intervals, generally two-week intervals, during a period of starvation. To prevent such worms from undergoing fission, it is only necessary to place them in dishes already well coated with slime from other worms.

The method of determining the oxygen consumption has already been described (1). Briefly the lot of worms was placed in a 500 cc. Erlenmeyer flask, filled air-tight with water of known oxygen content; after an interval a sample was drawn from this flask and its oxygen content determined. The difference between the oxygen content of this sample and the original oxygen content of the water gives the amount of oxygen consumed by the worms. The oxygen content was determined by Winkler's method. Two independent determinations were made each time. After the experiment was completed the worms were weighed. The temperature was the same each time the oxygen consumption of the worms was tested, but between these tests the worms remained at room temperature, which is naturally rather variable.

4. Results. The results of these tests on the oxygen consumption of five separate lots of P. maculata at various intervals during a period of starvation are summarized in table 1. The detailed data will be found at the end of the paper.

5. General conclusions regarding Planaria maculata. As shown in table 1, the oxygen consumption falls after feeding and reaches its lowest level within three or four days. It then remains at this level until after the second week of starvation. From this time on it rises continuously. A marked difference was found between the two varieties used. In the striped variety the oxygen consumption reached a maximum by the end of the sixth week of starvation and exhibited no further rise within the limits of the experiments. It is therefore not safe to begin these tests on worms which have already starved several weeks, as done by Allen in his experiment 4. In the spotted variety, on the other hand, the oxygen consumption continued to increase with an acceleration throughout the experiments. The experiment was discontinued in each case when the size of the worms was so reduced as

TABLE 1

Oxygen consumption of the spotted and the striped varieties of Planaria maculata during starvation, showing increase in all cases. All temperatures 20 ± 0.5°C.

TIME SINCE FEEDING	STRIPED VARIETY		SPOTTED VARIETY		
	Lot 1	Lot 10	Lot 3	Lot 2	Lot 4
	10-12 mm. (Original length)	8-12 mm. (Original length)	8 mm. (Original length)	8 mm. (Original length)	10-12 mm. (Original length)
	Oxygen consumed by 0.5 gram in two hours				
	cc.	*cc.*	*cc.*	*cc.*	*cc.*
1 day	0.18		0.25		
3-4 days	0.16		0.21	0.28	0.21
1 week	0.16		0.21		
2 weeks	0.15	0.22	0.22	0.28	0.21
4 weeks	0.17	0.26	0.24	0.29	0.28
6 weeks	0.22	0.35	0.29	0.36	0.30
8 weeks	0.21		0.29	0.59*	0.42
10 weeks	0.23*		0.52*		0.61*
Per cent increase...........	53	59	147	110	190

* Means that the worms were decapitated on the day preceding the test so that movement was eliminated.

to render further determinations impracticable. In most cases the heads of the worms were removed preceding the last test so that movement is eliminated as a factor in the result. In the early stages of the experiments, some of the stocks were not tested as this was considered unnecessary in view of the fact that the fall in oxygen consumption during this period is so well established that it may be assumed to have occurred. No tests are omitted from the table; all that were made are given.

EXPERIMENTS ON PLANARIA AGILIS

1. Source and care of material. This species is not indigenous to the Chicago region. About three hundred individuals were purchased from the firm of Powers Powers, Lincoln, Nebraska. Since Allen's stock was also obtained from this firm, we are dealing here with the same species. This species is stated by Mr. Powers to live in springs, a habitat similar to that frequented by P. dorotocephala. The two species resemble each other very much in appearance, although agilis is more restless and attains a larger size.

The original individuals were cut into pieces in order to obtain a sufficient number of individuals for the experiments. The experiments were not begun until three or four months after this had been done so that it is impossible that this procedure could have affected the results. The worms were fed by the same method as used for P. maculata and ate voraciously and grew with remarkable rapidity.

2. Digestive tract of Planaria agilis. In order to make a comparison between Planaria agilis and Planaria dorotocephala it is not sufficient, as Allen has done, to perform a few isolated tests. The physiology of the species must be studied before such comparisons are valid. One of the striking differences between these two species concerns the capacity of the digestive tract. Planaria agilis is an extremely voracious feeder, and appears to be very hungry after only three or four days' starvation. It will attack injured worms much more fiercely than will the other species we have observed. After it has fed, the body is noticeably distended so that it is quite easy to distinguish the individuals that have fed from those that have not. This observation suggested that the digestive tract has a larger capacity in P. agilis than in P. dorotocephala. Sections show that such is the case. Figure 1 gives camera lucida outlines of the body wall and digestive tract at various levels of section in individuals of P. agilis and P. dorotocephala of the same length. It is readily seen that the digestive tract of P. agilis, particularly in regions through and in front of the pharynx, is more capacious than that of P. dorotocephala. In the latter species, the anterior part of the digestive tract has the form of a median trunk with lateral branches, while in the former species there is a large irregular cavity extending nearly the width of the body. By simply distending the whole body, the capacity of this digestive tract would become very great and as already noted such distension occurs when P. agilis feeds.

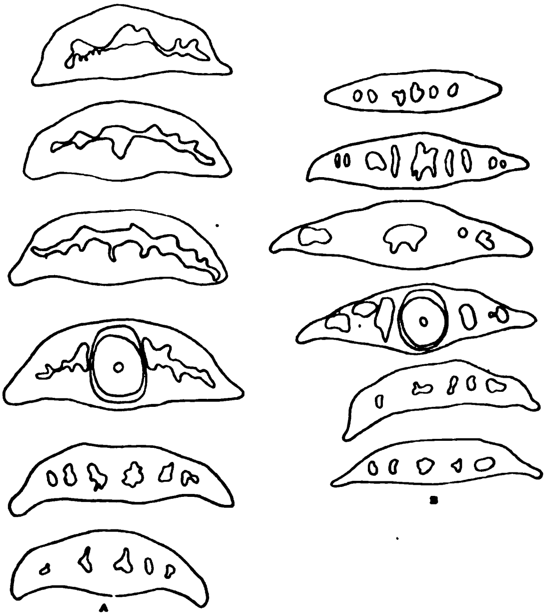

Fig. 1. *A*, six sections through Planaria agilis, drawn with the camera lucida, showing outlines of the cavity of the digestive tract; most anterior section at the top and others in order. *B*, sections through similar levels of Planaria dorotocephala, showing outlines of the cavity of the digestive tract. Both worms 15 mm. long.

Further the body is broader and thicker in P. agilis in proportion to length than in P. dorotocephala. These facts indicate that P. agilis can consume more food at each feeding and accumulates more food reserves than P. dorotocephala; and therefore it must be starved for a longer period before it will reach the same metabolic condition that the other species reaches in a short time.

3. Loss of weight of Planaria agilis as compared with P. dorotocephala. It was noticed that during starvation the reduction in size is much slower in P. agilis than in P. dorotocephala. To obtain a more accurate measure of this difference fifty worms of the same length of each species were weighed at intervals during starvation. Both lots of worms were kept under the same conditions throughout. The data on the loss of weight are given in table 2 and a graph constructed from the same data presented in figure 2. It is evident that P. doroto-

TABLE 2

Comparison of loss of weight of Planaria dorotocephala and Planaria agilis during starvation. Figures are weights of fifty worms, approximately 15 mm. long at the beginning of the starvation period

TIME SINCE FEEDING	PLANARIA DOROTOCEPHALA		PLANARIA AGILIS	
	Weight	Total decrease	Weight	Total decrease
	gram	*per cent*	*gram*	*per cent*
1 day	0.411		0.550	
1 week	0.322	21	0.503	8
2 weeks	0.284	30	0.472	13
4 weeks	0.214	47	0.380	30
6 weeks	0.159	61	0.289	47
8 weeks	0.107	74	0.227	58
10 weeks	0.073	82	0.169	69

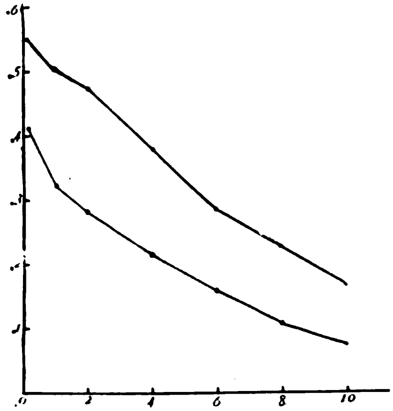

Fig. 2. Graph showing loss of weight of Planaria agilis and Planaria dorotocephala in starvation. Abscissa, time in weeks; ordinate, weight in tenths of a gram. Upper line, P. agilis; lower line, P. dorotocephala; weights for fifty worms.

cephala loses weight in starvation more rapidly than P. agilis and that the difference is greatest in the early part of the starvation period. The loss of weight of P. agilis is practically directly proportional to time so that the resulting graph is a straight line, as Allen has also shown. In P. dorotocephala on the other hand the loss of weight is more rapid at first and proceeds at a uniform rate only later in the starvation period. The slower loss of weight in P. agilis is evidently correlated with its greater breadth and thickness and greater supply of food reserves. Since P. agilis loses weight more slowly than P. dorotocephala, a longer time will be required for it to starve to the same degree and reach the same metabolic condition as the latter species. This was found to be the case.

4. Oxygen consumption of P. agilis during starvation. The methods employed were the same as those described under P. maculata. Five lots of worms, the members of each lot of approximately the same size, were isolated from the general stock and their oxygen consumption determined at various intervals after the cessation of feeding. Fission can readily be prevented in such lots by keeping them in dishes or pans coated with slime. A few fissions will occur and such divided worms were removed. The results of these five experiments are tabulated in table 3. The detailed data are given at the end of the paper.

The results tabulated in table 3 prove that in Planaria agilis as in other species of Planaria the rate of oxygen consumption increases during starvation. A longer period of starvation must elapse, however, in this species before the rise occurs. This length of time required depends, among other factors, on the original size (age) of the worms. Thus in lot 6, where the worms were 10 to 12 mm. long at the beginning of the experiment, the rise is present after four weeks of starvation; in lot 7, with worms 12 to 15 mm. long, the rise begins by the sixth week and in the other lots, with worms 15 mm. long or longer, the rise begins by the eighth week of starvation. In all cases the rise is plainly present by the beginning of the eighth week of starvation. This result is therefore contradictory to the contentions of Allen, who insists that within the length of time tested by him, namely, nine weeks, no such rise is present. I have already pointed out that his contention is directly contradicted by his own data given in his table 7, and that the results in this table are correct.

After the eighth week the rise is at first rather slow or even absent as in experiments 6 and 5 but later the increase is very rapid, and the oxygen consumption finally attained may be more than 100 per cent

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greater than the oxygen consumption after a starvation period of one week. After twelve or more weeks of starvation worms of this species are no further reduced in size than are individuals of Planaria dorotocephala of the same original length after six or seven weeks of starvation. The slower rate of reduction of P. agilis explains why the metabolic rate increases more slowly.

TABLE 3

Oxygen consumption of Planaria agilis during starvation, showing increase in all cases by the eighth week

TIME SINCE FEEDING	LOT 6	LOT 7	LOT 5	LOT 8	LOT 9
	18°C.	21°C.	18°C.	21°C.	21°C.
	10–12 mm. (Original length)	12–15 mm. (Original length)	15–18 mm. (Original length)	15–18 mm. (Original length)	18–20 mm. (Original length)
	Oxygen consumed by 0.5 gram in two hours				
	cc.	*cc.*	*cc.*	*cc.*	*cc.*
1 day	0.16	0.24	0.16	0.20	0.23
4 days	0.14		0.14		
1 week	0.14		0.13		
2 weeks	0.12	0.19	0.12	0.17	0.17
4 weeks	0.14	0.18	0.14	0.17	0.16
6 weeks	0.15	0.19	0.12	0.16	0.16
8 weeks	0.17	0.21	0.15	0.18	0.18
10 weeks	0.17	0.23	0.17	0.20	0.19
12 weeks	0.17	0.32	0.17	0.33	0.27
16 weeks	0.31*		0.25*		
Per cent increase......	158	77	109	106	65

* Means that heads were removed the day preceding the test.

5. Susceptibility of P. agilis during starvation. This matter has already been considered in the introduction. If the susceptibility method is reliable as a means of determining general metabolic rate, the susceptibility of P. agilis should increase more slowly during starvation than is the case with Planaria dorotocephala. I have made some tests of the susceptibility to cyanide of P. agilis during starvation. These tests were not as complete as is desirable but they indicated clearly enough the general situation in this species. All starving worms are more susceptible to cyanide than recently fed ones. Worms starved two weeks to five weeks are slightly more susceptible than those starved one week. There is, however, *no difference or very little difference in the susceptibility of worms starved two, three, four and five weeks.* Thus between the second and sixth weeks of starvation there is very little increase in susceptibility while during this period in P. dorotocephala a marked increase is demonstrable. Reference to table 3 shows that during this period also the oxygen consumption of P. agilis remains practically stationary. In later periods of starvation the susceptibility of P. agilis increases.

There is thus in this species a general agreement between the results by the susceptibility method and the results from direct measurement of the rate of oxygen consumption.

6. Comparison of starved and young (small)) individuals of P. agilis. It is insisted by Allen in his paper that whereas small worms consume more oxygen per unit weight than large ones, worms reduced by starvation consume the same amount of oxygen as before such reduction. This statement appears improbable on the face of it and my experiments show it to be erroneous. Small worms whether their size is due to youth or starvation consume more oxygen per unit weight per unit time than large worms in an adequate state of nutrition. It requires some twelve weeks of starvation to reduce P. agilis from a length of 18 to 20 mm. to a length of 6 mm. Such a reduction involves an increase of at least 60 per cent in the rate of oxygen consumption. According to Allen the difference in oxygen consumption between 6 mm. and 18 mm. worms is about 60 per cent (his table 10) when movement is not eliminated and about 40 per cent (his table 11) when

TABLE 4

Comparison of the rates of oxygen consumption of small (young) recently fed worms, large (old) recently fed worms, and worms reduced by starvation to a size similar to that of the young worms. Temperature 21°C.

KIND OF WORM	LENGTH	TIME SINCE FEEDING	OXYGEN CONSUMED BY 0.5 GRAM PER 2 HOURS
	mm.		
Young	6–10	5 days	0.22
	6–10	5 days	0.21
Old	15–18	2 weeks	0.17
	18–20	2 weeks	0.17
Starved	6	12 weeks	0.33
	6	12 weeks	0.27

movement is eliminated. As young fed worms move about much more than greatly starved worms, the latter figures are more correct.

In table 4 are given some data on the oxygen consumption of two lots of young worms 6 to 10 mm. long, starved five days; the oxygen consumption of worms 15 to 20 mm. long, from the same stock, starved two weeks; and the oxygen consumption of the same worms after reduction to a size similar to that of the young worms. After such reduction the worms have a higher rate of oxygen consumption than the young worms of similar size.

DISCUSSION

It is shown in this paper that in two varieties of Planaria maculata (which are probably separate species) and in Planaria agilis the rate of oxygen consumption per unit weight is increased by starvation. This increase begins in the first named forms within four weeks from the cessation of feeding but in Planaria agilis not until the eighth week if the original length exceeded 15 mm. This result agrees with results previously presented on other species (1) and disagrees with the contentions of Allen (2). Planaria agilis differs from all other species which have been adequately tested in that the oxygen consumption remains at nearly the same level between the second and sixth weeks of starvation, and rises only subsequently to this period. This circumstance is due to the fact that Planaria agilis starves more slowly than other species; it has a lower metabolic rate, greater food reserves, and a more capacious digestive tract, and consequently reduces more slowly in starvation than other species of Planaria. The remark of Allen that "the fact that the rate of oxidations is uniform during a long period of starvation will serve as an important basis for the study of respiratory metabolism in these forms" applies to Planaria agilis only. There is no level of "basal" or "standard" metabolism in other species, but their metabolism is continuously changing during starvation. Even in Planaria agilis the rate of oxygen consumption is probably not stationary but is falling very slowly during the first half of this period of apparent constancy and rising very slowly during the latter half of it.

Since Allen's paper is directed mainly against the reliability of the susceptibility method, it remains to make some statements regarding this method. In the first place, the susceptibility method measures the time of death. The time of death can naturally be determined only for those parts of the organism visible to the observer, namely, the superficial parts. Strictly speaking, therefore, survival time is a *measure of the metabolic rate of the body surface only*. That we have not in earlier publications emphasized this is due simply to the circumstance that the occasion for emphasizing it had not arisen. It had always been recognized by us that the susceptibility method concerns chiefly superficial structures and statements were made in various publications that internal systems and organs may show different susceptibility relations from the body surface.

It is, however, evident that *when special internal conditions are eliminated* survival time is a measure of general physiological and metabolic conditions in the whole organism. In the lower organisms there is only one such special internal condition which it has thus far been necessary to consider—that is the physiological condition of the digestive tract. When this factor is eliminated by keeping it in the same functional state in organisms which are to be compared then, as far as our data at present go, there is never any discrepancy between the results by the susceptibility method and the determinations of total oxygen consumption and carbon-dioxide production. A summary of some physiological conditions in which both susceptibility and respiratory rate have been determined is given in table 5.

From table 5 it will be seen that in nearly all cases where the survival time is shorter, the rate of respiratory exchange is greater, and where the survival time is lengthened, the rate of respiratory exchange is decreased, the sole exceptions being those concerned with feeding. That this apparent discrepancy exists was known to us for a considerable time before Lund and Allen published their work. Their viewpoint, that because this discrepancy exists the whole conception is erroneous, is certainly unscientific, to say the least. The correct procedure is to attempt to discover the cause of the discrepancy. This has been done and it has been found that the discrepancy is due to the behavior of the digestive tract. The respiratory rate of the digestive tract is increased by feeding but as the surface of the body is not affected by the feeding process, the susceptibility of the surface does not change. On the other hand the susceptibility of the intestine is increased by feeding, although it is somewhat difficult to observe the digestive tract by the susceptibility method in Planaria. In more transparent forms, like Hydra, the region of the body where food is being digested exhibits a very striking increase in susceptibility. After feeding, the respiratory rate of the digestive tract falls and as the body surface is again not involved in this process, the susceptibility of the surface is not altered. It is therefore necessary under circumstances involving the digestive tract to consider the body wall and digestive tract separately. As this is continuously done in physiological experiments on higher animals, where in order to study one physiological process it is necessary to have the others constant, we fail to see that such a procedure is "damaging to the theory that susceptibility is a measure of rate of oxidations," as Allen states. The method of determination of total metabolism in which Allen places such faith also fails to give any information about specific processes in the body unless other factors are controlled and eliminated. If one wants to measure the respiration in muscular work one cannot at the same time give the experimental animal a meal.

TABLE 5

Summary of the results from measurements of the survival time in toxic solutions and direct measurements of respiratory rate under different physiological conditions in Planaria

PHYSIOLOGICAL CONDITION	SURVIVAL TIME	OXYGEN CONSUMPTION	CO_2 PRODUCTION
Age (size)	Shorter the smaller (younger) the worm	Greater the smaller the worm	Same as for oxygen consumption
Injury	Shorter in injury	Greater in injury	Same
Regeneration	Shorter in regeneration	Greater in regeneration	Same
High temperature	Shorter	Greater	Same
Low temperature	Longer	Less	Same
Movement	Shorter than in rest	Greater than in rest	Same
In acid solution	Longer	Less	
Different levels	Shorter in anterior levels	Greater in anterior levels	Same
Late starvation	Shorter	Greater	Same
Feeding	No change in body surface	Greater	Same
Early starvation	No change or shorter in body surface	Less	Same

It may then be reiterated that as far as our experiments go, the survival time in toxic solutions is in simple animals a measure in general of the metabolic rate, as long as internal conditions remain constant. Survival time may not be an accurate measure of total respir-

TABLE 6

Detailed data on the oxygen consumption of Planaria maculata during starvation. Final calculation per unit weight per unit time in table 1

TIME SINCE FEEDING	LOT 1		LOT 2		LOT 3		LOT 4		LOT 10	
	Oxygen consumed	Weight	Oxygen consumed	Weight	Oxygen consumed	Weight	Oxygen consumed	Weight	Oxygen consumed	Weight
	cc.	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*
1 day	0.54 (1½ hrs.) 0.56	1.833			0.43 (1½ hrs.) 0.55	1.277				
4 days	0.43 (1½ hrs.) 0.43	1.667	0.54 (2 hrs.) 0.47	0.874	0.36 (1½ hrs.) 0.36	1.110	0.46 (2 hrs.) 0.49	1.104		
1 week	0.41 (1½ hrs.) 0.36	1.584			0.33 (1½ hrs.) 0.37	1.046				
2 weeks	0.42 (2 hrs.) 0.36	1.222	0.37 (2 hrs.) 0.34	0.634	0.42 (2 hrs.) 0.36	0.862	0.38 (2 hrs.) 0.36	0.818	0.36 (3 hrs.) 0.35	0.521
4 weeks	0.28 (2 hrs.) 0.25	0.764	0.23 (2 hrs.) 0.19	0.362	0.30 (2 hrs.) 0.27	0.579	0.31 (2 hrs.) 0.23	0.474	0.15 (3 hrs.) 0.17	0.205
6 weeks	0.29 (2½ hrs.) 0.28	0.507	0.19 (3 hrs.) 0.15	0.149	0.24 (2½ hrs.) 0.22	0.298	0.21 (3 hrs.) 0.19	0.215	0.19 (6 hrs.)	0.093
8 weeks	0.18 (3 hrs.) 0.16	0.258	0.11 (3 hrs.) 0.11	0.062	0.15 (3 hrs.) 0.14	0.166	0.14 (3 hrs.) 0.14	0.111		
10 weeks	0.08 (3 hrs.) 0.06	0.098			0.12 (3 hrs.) 0.10	0.065	0.16 (6 hrs.)	0.043		

TABLE 7

Detailed data on the oxygen consumption of Planaria agilis during starvation. Final calculation per unit weight per unit time in table 8

TIME SINCE FEEDING	LOT 5		LOT 6		LOT 7		LOT 8		LOT 9	
	Oxygen consumed	Weight	Oxygen consumed	Weight	Oxygen consumed	Weight	Oxygen consumed	Weight	Oxygen consumed	Weight
	cc.	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*	*cc.*	*grams*
1 day	0.51 (1½ hrs.) 0.54	2.196	0.60 (1½ hrs.) 0.62	2.417	0.80 (2 hrs.) 0.82	1.632	0.84 (2 hrs.) 0.82	1.755	0.95 (2 hrs.) 0.96	2.062
4 days	0.39 (1½ hrs.) 0.38	1.730	0.48 (1½ hrs.) 0.56	2.175			0.46			
1 week	0.34 (1½ hrs.)* 0.27	1.500	0.50 (1½ hrs.) 0.39	2.031						
2 weeks	0.35 (2 hrs.) 0.29	1 329	0.50 (2 hrs.) 0.42	1.827	0.41 (2 hrs.) 0.38	1.022	0.46 (2 hrs.) 0.42	1.275	0.44 (2 hrs.) 0.41	1.188
4 weeks	0.37 (2 hrs.) 0.27	1.083	0.50 (2 hrs.) 0.36	1.469	0.31 (2 hrs.) 0.28	0.812	0.39 (2 hrs.) 0.34	1.052	0.46 (2 hrs.) 0.40	1.289†
6 weeks	0.27 (2½ hrs.) 0.28	0.871	0.41 (2½ hrs.) 0.36	1.018	0.32 (3 hrs.) 0.32	0.558	0.38 (3 hrs.) 0.34	0.725	0.42 (3 hrs.) 0.38	0.828
8 weeks	0.33 (3 hrs.) 0.30	0.667	0.43 (3 hrs.) 0.37	0.749	0.27 (3 hrs.) 0.24	0.394	0.29 (3 hrs.) 0.25	0.488	0.33 (3 hrs.) 0.30	0.557
10 weeks	0.25 (3 hrs.) 0.24	0.480	0.28 (3 hrs.) 0.25	0.510	0.19 (3 hrs.) 0.21	0.288	0.20 (3 hrs.) 0.22	0.346	0.21 (3 hrs.) 0.25	0.338
12 weeks	0.17 (3 hrs.)	0.322	0.20 (3 hrs.) 0.16	0.336	0.28 (5 hrs.)	0.173	0.29 (5 hrs.)	0.202	0.33 (5 hrs.)	0.248
16 weeks	0.15 (5 hrs.)	0 093	0.18 (5 hrs.)	0.143						

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* The higher rate of oxygen consumption usually found in the first of each pair of determinations is due to movement. The amount of movement is greatest in the middle of the starvation period. It was later eliminated to some extent by placing the worms in the experimental flasks the day before the test.

† Worms of the same kind added.

atory rate because other factors than respiratory rate may be concerned in survival time. Argument over the reliability of the susceptibility method has now been rendered unnecessary because all of the main conclusions drawn by the use of this method have been verified by direct determinations of oxygen consumption and carbon-dioxide production.

SUMMARY

1. This paper is concerned with the rate of oxygen consumption during starvation of Planaria maculata and Planaria agilis and was undertaken in reply to a paper by Allen (1).

2. Contrary to the results of Allen it was found that in both of these species within the time limits specified in his paper, the rate of oxygen consumption is increased in the later periods of starvation. His data are criticised and it is pointed out that they are with one exception inadequate and unsatisfactory; the one exception contradicts his own conclusion.

3. Planaria agilis has a lower metabolic rate, greater food reserves, and loses weight more slowly in starvation than other species. For this reason it must be starved for a longer period before its rate of oxygen consumption increases.

4. Owing to the slow rate of starvation of Planaria agilis, there is a period from the second to the sixth week of starvation when the oxygen consumption is nearly constant. This is not the case in other species.

5. The susceptibility of Planaria agilis to potassium cyanide also fails to increase during starvation as rapidly as in other species. Contrary to the contentions of Allen, the susceptibility results in this species are then in general agreement with the results from direct measurement of the oxygen consumption.

6. Contrary to the statements of Allen, it was found that in Planaria agilis worms reduced to a small size by starvation have as high a rate as or a higher rate of oxygen consumption than small recently fed worms of the same size.

7. A general discussion of the utility of the susceptibility method as a measure of metabolic rate is given and it is pointed out that no discrepancies are at present known to us between the results by the susceptibility method and the results by direct determination of respiratory rate, except those concerned with feeding. In the case of feeding, it is necessary to draw a distinction between the metabolism and susceptibility of the digestive tract and those of the body wall.

8. The results in this paper further support the general conception maintained by Child for years that the metabolism of organisms is increased in starvation and that starvation is therefore a means of bringing about rejuvenescence.

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VASOMOTOR REFLEXES FROM RECEPTOR STIMULATION IN INTACT ANIMALS

E. G. MARTIN, A. C. FRANKLIN AND CLARENCE HIELD

From the Laboratory of Physiology, Stanford University

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Of recent years the investigation of vasomotor reflexes in mammals other than man has been carried on chiefly on the basis of artificial stimulation of nerve trunks, rather than by means of receptor stimulation; the older physiologists, on the other hand, gave much attention to the effects on blood pressure of excitations applied to the receptors themselves. The latter method would appear to be the more logical if the object sought is to determine the normal mode of functioning of the vasomotor apparatus, although where the underlying purpose is the study of reflex action, with the vasomotor reflexes selected as examples of such action, direct stimulation of sensory nerve trunks is not only valid, but has much to recommend it, both in theory and practice.

One of us (M.) has been for some years interested in vasomotor reflexes, not so much from the standpoint of their significance as regulating factors of the circulation as for the light they may throw on the reflex functioning of the nervous system itself ((1) to (6)). In all the experiments described in the above series of references the elicitation of reflex responses was by means of stimulation of nerve trunks. Certain of the findings therein have suggested the desirability of repeating some of the experiments of the older investigators on the reflex vasomotor effects of *receptor* stimulation, with the attention specially directed to the bearing of such experiments on theories of nervous action.

PROCEDURE

Our plan of study called for the elicitation of vasomotor reflexes in conscious and in very lightly narcotized animals, as well as in decerebrate and in deeply anesthetized individuals. It followed that the method of recording the responses must be applicable to conscious animals. Blood pressure determinations were ruled out because of the operative procedures involved; the choice was thus restricted to plethysmographic methods, or to such as use temperature variations as indices of vasomotor changes. Either of these latter have the disadvantage of showing only local effects, as contrasted with the general influences revealed by blood pressure determinations. A vasomotor response that is restricted to the splanchnic region might escape detection completely under such circumstances. This is a limitation that is unavoidable, however, in experimentation on conscious animals. If the site of application of the recording device is fortunately chosen it should reveal any vasomotor change that affects cutaneous areas. One important desideratum is that the region selected shall be known to have both constrictor and dilator innervation, since interpretation of the interaction of dilator and constrictor responses is a significant feature of the general problem (5).

Following the example of Grützner and Heidenhain (7), our experiments were made on rabbits. The varieties were those widely raised in California for market purposes, including some common domestic rabbits and some Belgian hares. On the whole we found the latter less satisfactory than the smaller and hardier rabbits. We experimented at some length with the nasal cavity as a plethysmograph, using the method of Tschalussow (8) as modified by Mendenhall (9) but found it unsuited for work with rabbits. We then turned to the ear as a field of study. Since in rabbits the ears play an undoubted, and probably considerable, part in temperature regulation (10), they might be expected to be as sensitive to vasomotor influences as any of the cutaneous areas. That they have dilator as well as constrictor innervation appears from the work of Winkler (11), cited by Bayliss (10).

To record vasomotor changes in the ear we used an ordinary air plethysmograph, communicating with a sensitive tambour, whose lever wrote on a slow drum. The air plethysmograph offers a double advantage for work of this kind; in addition to being itself a sensitive recorder of volume changes in the ear, it responds also to changes of temperature; vasodilatation would bring about both an increased ear volume and a higher ear temperature; the latter by causing expansion of the air in the plethysmograph would add its effect to the increase of ear volume, enlarging the tracing on the drum. Obviously sudden changes in temperature due to external influences had to be guarded against, but this was a simple matter.

For keeping unanesthetized rabbits in position we used a board 6 inches wide, mounted on a pedestal about a foot high. This board was cut away at front and rear, so that when a rabbit was laid on it his legs would hang down comfortably. A couple of short straps were buckled around the board and over the back of the animal, and a rest provided for his chin. With this arrangement a rabbit would remain quiet for two or three hours, making few movements, except when stimulated thereto in connection with our experimentation. At first we tied the head firmly, but this proved unnecessary and since it often seemed to impede the blood flow through the ears was abandoned. A precisely similar arrangement was employed with anesthetized or decerebrate animals, except that with most of these we added a head-clamp. The hair was regularly clipped from all four legs, and from a considerable area of the back.

Stimulations. Tactile, thermal and auditory stimuli were used with success; visual stimuli, in the form of light flashed on and off before the eyes, gave negative results so far as vasomotor manifestations are concerned.

For tactile stimuli we followed the practice of Grützner and Heidenhain (loc. cit.) and used blowing upon the skin, rubbing the mouth, inside, and upon the lips, with a hard instrument, and pulling at individual hairs with forceps, all gentle stimuli and, according to the experience of those investigators, highly effective in evoking vasomotor reactions. We found the free ear the most favorable spot on which to blow.

Several sorts of thermal stimuli were used. Dipping a leg into water of selected temperature for a measured time, thirty seconds to two minutes, proved moderately satisfactory. The leg, as stated above, was clipped of hair, and was thoroughly greased with vaseline before dipping into water. We used a container holding a liter; the temperature of the relatively large volume would remain fairly steady during the period of immersion. The position of the animal on the holder permitted the bringing of the vessel of water up around the leg without disturbing the experiment. The range of temperatures employed will be given further along, in connection with the detailed account of the results of the experiments. A second method of thermal stimulation, of which we made much use, consisted of placing an electric light, backed by a conical reflector of ordinary type, a short distance above the back, from which, as noted above, the hair had been clipped. To insure that the rays from the light should not strike upon the plethysmograph directly, and so warm the air within it, we interposed a double screen; this consisted, first, of a large sheet of cardboard placed upright between the rabbit's back and head, and cutting off from the whole head and its surroundings any direct rays from the light; and second, of a cylinder of sheet asbestos, about double the diameter of the plethysmograph, placed about it. A sensitive thermometer inserted between the asbestos cylinder and the plethysmograph showed that no change of temperature occurred upon turning on the light. Another sensitive thermometer was laid along the animal's back, with the bulb in close contact with the skin, to enable some idea to be formed of the rate of warming of the back, as well as the maximum temperature reached. Since it was probable that the temperature of the thermometer bulb would rise faster than that of the skin, where the bulb was freely exposed to the light, we protected the bulb, in some of the experiments, with a small bit of asbestos. This precaution was not wholly satisfactory, since it was now probable that the skin in contact with the thermometer bulb, being protected with asbestos, as well as by the bulb itself, would warm up more slowly than areas fully exposed to the light, and so introduce an error in the opposite direction. To cool the skin of the back we placed upon it a cloth bag of cracked ice, or obtained the desired effect by pouring a small quantity of ether upon it.

Various auditory stimuli were tried, but the only really satisfactory one consisted of a shrill whistle, blown near the ear of the rabbit, but with precaution not to allow the blast of air to strike the animal.

VASOMOTOR RESPONSES IN NORMAL RABBITS

For experiments on conscious animals we were obviously limited to stimuli which would be endured without struggling, since quiet was essential to the securing of a convincing record. This meant the confining of the stimulation, in general, to the levels below the threshold of pain, or the selection of stimuli which have no marked painful quality, even when intense. The investigation became, in a sense, a comparison of the thresholds of vasomotor and skeletal muscle reflexes, since our practice was to push the stimulation, whenever possible, until the animal began to struggle, observing meanwhile such changes in ear volume as occurred.

Tactile stimuli. Confirming the findings of Grützner and Heidenhain (7) that gentle stimulation is effective in arousing vasoconstriction, we obtained repeated shrinkage of ear volume by blowing with the breath on the exposed ear, or the back (clipped of hair) or the hind leg, or the nose. The onset was prompt; the maximum effect was reached within fifteen seconds, and the return to former volume was completed within thirty to forty-five seconds. Contact of an ice pack with the back caused, on several occasions, an ear shrinkage that was obviously due to the contact and not to any lowering of temperature, since the onset was as prompt as in the blowing experiments, and recovery occurred as quickly. Localized irritation, as by pulling at a small group of hairs with forceps, did not produce any demonstrable change in ear volume.

A feature of these responses which we wish to stress is that they were definitely more pronounced early in the course of the experiments than later. There appeared to be a habituation or fatigue which operated to cut down their magnitude. To illustrate: a large black male rabbit, which had been experimented on on several previous occasions, gave, within the first five minutes of the experiment of February 19, 1920, typical responses to blowing upon the ear. Ten minutes later the response was still evocable, but much reduced; a half-hour later the same sort of stimulation failed to elicit any response. Throughout the period stimuli of one sort or another were applied every minute or two. The animal was then removed from the apparatus and allowed to run about the floor for ten minutes. It was then replaced in position for recording ear volume changes and similar stimuli applied. Shrinkage of ear volume occurred, comparable with that obtained at the very beginning of the experiment; again, however, there was a diminution of effect, so that at the end of fifteen minutes the response was no longer obtainable.

Auditory stimuli. Shrill whistling, prolonged for five or six seconds, brought about ear shrinkage very much like that obtained from tactile stimulation. The latency was short, and the duration about equal to that of the responses to blowing on the ear or back. To assure ourselves that the effect on the plethysmograph was actually due to a change of ear volume, and not to a movement of the tympanic membrane, we stuffed the external auditory meatus with vaseline; this procedure did not change the response. These observations are in accord with those of Dogiel (12). We observed a habituation to auditory stimulation similar to that reported above for tactile. The vasoconstriction from whistling was frequently greater at first than the maximum from blowing on the back or ear, but quickly became less, and complete failure occurred earlier. A short rest outside the apparatus restored the effectiveness of the stimulation momentarily.

Thermal stimuli. We attempted to lower the temperature of the skin of the back by laying an ice pack on it and by pouring ether on it. In spite of repeated attempts the first method failed to yield demonstrable vasoconstriction in conscious rabbits. The animal would endure the presence of the ice pack for about two minutes, but would then begin to struggle so violently that the pack had to be removed. No change in ear volume occurred during the two minutes in which the pack was in contact with the back, except for the transitory vasoconstriction due to contact described above. The chief interest of this observation lies in the apparent demonstration that cooling of the back becomes sufficiently uncomfortable to arouse the conscious animal to definite efforts at escape before the stimulation of cold reaches the point of evoking demonstrable vasoconstriction. We were uniformly successful in obtaining vasoconstriction when ether was poured on the back. The effect developed promptly, reached a maximum within a half-minute, and usually passed off within two or three minutes. A small volume of liquid, roughly 1 cc., gave the best results. On one occasion we tried pouring about 5 cc. on the back. Vasoconstriction began to develop in the usual way, but before it had attained maximal extent the animal displayed signs of annoyance at the odor, and presently began to struggle. We attribute the constrictor effect induced by ether to the cooling brought about by the rapid evaporation, and we have evidence from the thermometer placed against the back that there was prompt and marked cooling; on two occasions the back temperature fell eight degrees in two minutes. The possibility that the ether may have had an irritating effect apart from the cooling is not excluded, although no marked irritation is experienced from contact of ether with the human skin. It is possible that our superior success with ether as compared with the ice pack was due to a degree of fright induced by the strange odor, which kept the animal quiet except in the case where an excessive dose was used, when the desire to escape became paramount.

Vasodilatation (swelling of ear) was readily obtained in conscious rabbits by warming the back with an electric light in the manner described above. The animals usually endured the warming for several minutes without showing signs of discomfort. In every trial but one of our series vasodilatation began to show itself before any skeletal muscle movements were made. In the one exceptional case there were signs of restlessness before vasodilatation became perceptible, but the movements were not extensive enough to necessitate discontinuance of the experiment, and increase in ear volume began to show itself before struggling became pronounced. There was considerable variation in the latency of the vasodilatation; in some cases the ear began to swell within a few seconds after turning on the light; in others two or three minutes elapsed before visible increase in ear volume appeared. In the exceptional case mentioned above, in which struggling preceded ear swelling, the warming continued for seven minutes before the bodily movements began; the swelling of the ear did not become demonstrable until eleven minutes after applying the electric light. As stated in a former paragraph, we do not consider the recorded changes in back temperature very significant because of the large probable error, inherent in the use of a mercury thermometer laid in contact with the skin. It is, however, perhaps worth noting that on four occasions the rise in skin temperature preceding the beginning of struggling on the part of the animal coincided at 6°.

VASOMOTOR REFLEXES IN DECEREBRATED RABBITS

Our method for preparing the rabbits was that usually employed in experiments involving the procedure of decerebration. Light ether anesthesia was maintained throughout the operation up to the completing of the cut across the brain stem. Clamps were applied to the carotids, and the vertebral arteries were occluded by pressure of the fingers and thumbs of an assistant at the sides of the vertebral column immediately behind the skull. In rabbits stoppage of the circulation through the vertebrals is easily accomplished in this manner. As soon as possible after completion of decerebration the pressure on the vertebrals was released and the clamps on the carotids removed.

Tactile stimuli. The only suggestions of vasoconstrictor reflexes following tactile stimulation were obtained within the first fifteen minutes after decerebration. On several occasions we noted what looked like extremely slight positive responses, but only once was there a reaction that at all approached in magnitude our usual responses to similar stimulation in normal rabbits. On this occasion the reaction followed laying the hand gently on the rabbit's head. About a minute later repetition of the stimulus was followed by vigorous galloping movements on the part of the animal. Perhaps it is worth noting that whenever our procedures aroused decerebrate rabbits to violent movements they took the form of galloping; this contrasts sharply with the struggling of conscious rabbits, whose effort was invariably to push themselves backward off the board on which they lay; an effort explicable when one recalls that the chief unaccustomed factor in the situation was the plethysmograph tube on the ear. In no case did tactile stimuli applied during the later stages of an experiment give positive vasomotor results. That this failure was not due to diminished sensitivity of the animals was shown by the presence during the same stage of the experiment of definite skeletal muscle responses to slight stimuli.

Auditory stimuli. We were unable to obtain a definite vasomotor reaction to shrill whistling in any instance, although skeletal muscle responses were readily elicitable in nearly all our trials. In one case the galloping activity, noted above as characteristic of violent effort in decerebrate rabbits, followed an auditory stimulus, although in most of our trials only slight movements of ear or head were noted. We wish to stress the point that two sorts of gentle stimulation, tactile and auditory, which in normal animals typically bring about well-marked vasomotor reflexes, fail almost completely in this regard in decerebrates, although skeletal muscle reflexes are obtained about as well in one form as in the other.

Thermal stimuli. We obtained typical vasoconstriction from pouring ether on the back of decerebrated rabbits; in appearance this corresponded fully with the response of normal animals to similar stimulation. Warming the back by means of an electric light gave vasodilatation (ear swelling) in all our trials except two. There appeared to be less tendency than in normal rabbits toward struggling; at least the animal would usually remain quiet for a longer time after the light was turned on than would the normals. On two occasions no ear swelling was manifest; on one of these there appeared to be a slight ear shrinkage. We carried out an extensive series of observations on the results of immersing a leg in water. As stated above, the leg was thoroughly greased with vaseline before immersion. A hind leg was used in most of our experiments. The front leg was tried, but in no case with positive results. Water at 1°C. (ice water) was used repeatedly but neither with decerebrates nor with rabbits in any other state did we ever obtain a perceptible vasomotor change therewith. For the tests with warm water temperature intervals of 5°C. were used, beginning with 35°. No changes in ear volume were observed at temperatures below 50°. At 50° vasodilatation occurred regularly; at 55° vasodilatation occurred in approximately half the trials and vasoconstriction in the other half; at 60° and 65° only shrinkage in ear volume was seen, except that on one occasion a slight increase in ear volume preceded the constriction. These observations suggest that there is a temperature point at which reversal of vasomotor effect occurs.

VASOMOTOR REFLEXES IN RABBITS UNDER LIGHT ETHER ANESTHESIA

In our experiments in which ether was used as the anesthetic, pains were taken to keep the narcosis as shallow as possible; some variation in depth of anesthesia was inevitable with the method of administration, which was by an ordinary nose cone. *Tactile stimuli* appeared to be less effective in eliciting vasomotor responses than in unanesthetized rabbits; at least such as we tried were ineffective, although they were pushed to the point of inducing struggling. Shrill whistling was followed by transient shrinkage of the ear; very definitely in the early stages of the experiment; less so in the later stages. It was our impression that there was a habituation to the stimulus, similar to that suggested by the experiments on conscious animals, but the possibility that increasing depth of narcosis might account for the lessened effect is not excluded.

Warming the back by means of an electric light resulted in vasodilatation; with very light narcosis the animal would endure the warming without struggling for only a few minutes, so that extended exposure to the warming could not be studied. Immersing a hind leg in water at 45°C. was followed in one instance by definite ear swelling, although shortly afterward temperatures of 49°, 50° and 51° gave negative results. Water at 52° brought about commencing vasodilatation, but almost coincident with the beginning of the change in ear volume the animal became restless and the hot water had to be withdrawn. When the leg was immersed in water at 55° the animal struggled so promptly and violently that no vasomotor record could be obtained.

VASOMOTOR REFLEXES IN RABBITS PARTIALLY ANESTHETIZED WITH URETHANE

We were much interested in the possible effects of urethane on the responses to receptor stimulation, since Martin and Lacey (1) had shown that the threshold of vasomotor reflexes from *nerve*-trunk stimulation is not very much raised by urethane. We performed our experiments with urethane in two series; in the first only half the standard dosage was used (1 gram per kilo body weight); in the second we used standard full dosage. The appearance of rabbits that have received only half the usual dose of the narcotic differs characteristically from that presented by fully anesthetized animals. They show a degree of prostration, but chiefly in the rear half of the body. The hind legs are frequently quite useless, but some control is retained over the front legs, and the neck muscles appear to be only slightly affected so that the head is held in about the usual position. This is in sharp contrast to the complete muscular relaxation of rabbits under the influence of full dosage.

Tactile stimuli. The same kinds of tactile stimuli were used as under the other experimental conditions employed in the investigation, namely, blowing on or pinching the ear, blowing on the back or leg, rubbing the inside of the mouth with a blunt instrument. In about half the trials ear volume showed slight decrease, indicating some vasoconstriction; in the others no change was seen. On two occasions pinching the ear brought about struggling, showing that skeletal muscle reflexes were rather readily evocable.

Auditory stimuli. In only one out of nine trials did whistling fail to elicit vasoconstriction; one of the results recorded as positive was so slight as to be questionable; all the others were definite.

Thermal stimuli. Cooling the back by pouring ether on it did not give as satisfactory results as in normal or decerebrated rabbits. We did not obtain in any instance as good a record of ear shrinkage from this procedure as appeared regularly in those series. The partially urethanized animals spoiled the record by struggling much more frequently, but in the few cases in which struggling did not occur clear-cut vasoconstriction was not manifested, although the record indicated its presence to a very slight degree.

Warming the back with an electric light was followed regularly by well-marked vasodilatation. The warming was ordinarily endured for only about four minutes; at the end of that time struggling would begin and the light have to be removed. There would have been time, meanwhile, for the ear swelling to show itself. Immersing a hind leg in warm water gave vasodilatation at 45°, 50°, and 52°; at the latter temperature the effect was very slight; possibly there was a habituation from the previous immersions at the lower temperatures. At 54° the animal struggled thirty seconds after applying the warm water; no swelling of the ear had developed at that time.

Observations on the late effects of urethane. We were interested in the late effects of urethane on the relative sensitiveness of the vasomotor and skeletal muscle reflex paths; accordingly we reëxamined our animals at the end of twenty-four hours, and in one case of a half-dose animal again after forty-eight, and after seventy-two hours. Our rabbits that had received only half the usual urethane dosage were in general appearance nearly over the effects of the drug on the day following administration. They ordinarily showed some lack of coördination in the movements of the hind legs, but in other respects behaved much like normal rabbits. Such skeletal muscle reflexes as attended our receptor stimulations were definitely more easily elicited than on the preceding day. In contrast to this near recovery of skeletal muscle control is the interesting fact that vasomotor reflexes were nearly inelicitable. Vasodilatation resulted from warming the back with an electric light, but all our other procedures were negative, except that in a single instance rubbing the inside of the mouth was followed by a minute curve in the tracing which may have been an indication of very slight vasoconstriction.

After forty-eight hours the single rabbit that was observed extensively presented, in all respects, the appearance of complete recovery. It responded to mechanical and auditory stimulation by vasoconstriction in precisely the fashion of normal rabbits. The only difference that we observed was that no ear shrinkage followed the pouring of ether on the back. To determine whether this response would be reëstablished we repeated the tests on the following day, namely, seventy-two hours after narcotization. In addition to positive results from all our other tests we now obtained definite vasoconstriction when ether was poured on the back.

VASOMOTOR REFLEXES IN RABBITS FULLY ANESTHETIZED WITH URETHANE

As stated above, rabbits under full urethane narcosis show complete muscular relaxation. In our experience this comes on quite promptly; it is accompanied by a pronounced slowing of the breathing. We made a number of counts of the respiratory rate before and after administering urethane, and obtained slowing from a preliminary rate ranging between 140 and 180 a minute to a rate of 60 to 70 a minute.

Tactile stimuli. We obtained definite changes in ear volume following tactile stimulation in about 70 per cent of our trials; since we experimented with various means of stimulation the percentage of negative results is probably not unduly large. Contrary to our experience with rabbits in other states as regards narcotization, in all of which mechanical stimulation gave only vasoconstriction, we observed in nearly a third of our positive trials in this series swelling of the ear (vasodilatation); in the remaining two-thirds the usual vasoconstriction was seen. In an attempt to account for the occurrence of contrary responses from similar stimulations we classified the tactile stimuli employed as gentle or harsh. To the first group were assigned stroking the skin, handling the ear gently, and blowing on the ear or back; in the second were included pinching the skin hard with fingers or forceps and rubbing the inside of the mouth with a blunt instrument; the free ear was the area most frequently selected for pinching. All our vasodilator responses but one resulted from the application of stimuli classified as gentle; 70 per cent of the constrictor responses, on the other hand, followed harsh stimulation.

Notwithstanding the complete muscular relaxation characteristic of fully urethanized rabbits skeletal muscle reflexes were not wholly absent. On three occasions mechanical stimulations were followed by struggling. Two of these were after harsh stimuli; the third occurred after blowing on the skin, a stimulus classified by us as gentle.

Auditory stimuli. Shrill whistling was followed by ear shrinkage (vasoconstriction) regularly in the early stages of the experiments. In only three early trials did we fail to get the response. In all these experiments stimulation was begun as soon as possible after signs of complete narcotization had developed. In only one case did we observe a positive effect as much as forty minutes after the beginning of experimentation. In general urethanized rabbits seemed to respond positively to auditory stimulation only during the very early stages of narcosis. In all our experiments of this series good responses to tactile stimuli were elicitable a half-hour or more after auditory stimulation had ceased to be effective. In two instances transient ear swelling followed the whistling; these were the only cases in our entire experience in which any other positive response than vasoconstriction followed auditory stimulation.

Thermal stimuli. Both our methods of lowering skin temperature, laying an ice pack on the back, and pouring ether thereon, brought about vasoconstriction repeatedly. We failed to get this result only twice, both times during the very late stages of the experiments. Raising the back temperature by means of an electric light was followed just as regularly by swelling of the ear (vasodilatation). In several instances the onset of the dilator reaction was considerably delayed. During this latent period we observed several times, but not invariably, a slight but perfectly definite ear shrinkage, which suggests the possibility of a local vasodilatation in the warmed area of the back which would cause an increased flow of blood through that region at the expense of other parts of the body. With the development of general reflex vasodilatation active swelling would succeed this passive shrinkage in such areas as the ear. On only two occasions did the warmth on the back lead to struggling. This contrasts sharply with the invariable occurrence of this reaction in normal rabbits.

Immersing one hind leg in warm water was followed by swelling of the ear (vasodilatation) in just half our trials; all the others were negative except two, in which there appeared to be a slight shrinkage of the ear. Positive vasodilatations were obtained at temperatures of 50° and upward; none were seen at 45°, although that temperature was tried repeatedly. We obtained in two cases ear swelling at 65°; subsequent tests at 70° and 75° were negative. We have reason to suspect that injury to the receptors occurred at 65°, since the leg that had been immersed in this very hot water was much swollen and blistered on the following day. For comparison it might be well to note that water at 50° to 52° is extremely painful to the human hand if immersion is maintained for as much as a minute, as we demonstrated upon ourselves. The observations which we interpreted as showing vasoconstriction occurred at 53° and 58°. There was struggling, sometimes violent, at nearly all temperatures above 53°. The latency was sufficient to allow the change in ear volume to show itself while the animal was still quiet. In one case the skeletal-muscle response to immersion of a hind leg in hot water took the form of definite running movements.

Late effects of full urethane narcotization. In an earlier section we described observations on the recovery from half doses of urethane. Similar observations were made on the late effects of full doses. At the end of twenty-four hours we found substantially the same situation with respect to vasomotor responsiveness that is recorded above for partially urethanized animals, namely, an almost complete insensitiveness. We have one observation of ear swelling following warming the back with an electric light, but with by far the longest latent period (thirteen minutes) seen in the investigation. We have also one record of vasoconstriction resulting from pouring ether on the back. All our other numerous tests, including all the kinds described in this paper, were negative. These rabbits showed much less progress toward recovery of skeletal muscle control than did those under half-urethane after a similar interval. They did, however, respond regularly by struggling to most forms of mechanical stimulation, to ether on the back, and to immersion of a hind leg in water at temperatures above 51°C. The respiratory rate was that of complete urethane narcosis, namely, 60 to 70 a minute.

A single rabbit that was examined at intervals until completely recovered gave the following history: at thirty hours after administration of urethane the breathing was still slow, 72 in the minute; there were no well-marked ear volume changes as the result of stimulation, although very small curves appeared in the tracings in connection with the application of some of the stimuli. An interesting feature of such as did appear was that with one exception they were of opposite sign to the usual responses; thus, both tactile and auditory stimuli gave what would have been interpreted as vasodilatation if the curves had been more pronounced, whereas the ordinary effect of such stimuli is definite vasoconstriction. One tactile stimulus did give apparent ear shrinkage, this being the single exception mentioned above. After forty-six hours the breathing in this rabbit had returned to the normal rate, 170 in the minute; there was still considerable muscular incoördination. The vasomotor responses to stimulation were more marked than after thirty hours, but again both auditory and tactile stimuli were followed by dilatation instead of by the usual constriction. Warming the back with an electric light gave dilatation; immersing a hind foot in warm water (45°–55°) was without effect on ear volume; at 55° the animal struggled. Fifty-four hours after anesthetization both tactile and auditory stimuli yielded vasoconstrictor reactions, although in five out of eight trials there was a slight preliminary dilatation preceding the constriction. Pouring ether on the back resulted at this time in the usual constrictor response. At the end of seventy-six hours the vasomotor reactions were in every respect similar to those previously obtained by us from unanesthetized rabbits.

DISCUSSION

The transient vasoconstrictor response obtained by us under mild tactile stimulation appears to be identical with that described by Grützner and Heidenhain (7) for a similar situation. The investigators just mentioned observed a marked heightening of the response under curare poisoning, and were so impressed therewith that their further study concerned itself with the reflex as exhibited by rabbits in curare paralysis, to the exclusion of its consideration in unpoisoned animals. In addition to their finding that the vasoconstriction was heightened in curare paralysis they showed that very severe stimulation of the cutaneous endings, instead of affording even more pronounced results, failed to induce any response whatever. Before discussing the features in which our observations agree with those of Grützner and Heidenhain a brief consideration of these curare effects seems desirable. The point about them which was chiefly stressed by the authors was that they appeared only at a certain dosage of curare, a fact which probably explains the failure of recent workers in the same field (Sollmann and Pilcher (13), Martin and Stiles (3)) to report the phenomena. To our mind this confinement of the effect to a certain stage of curare poisoning signifies an acute disturbance of nervous equilibrium due to the drug, and negatives the application of the observations to the interpretation of normal nervous functioning. We are not willing to accept, for this reason, the view of Grützner and Heidenhain that the marked rise of blood pressure seen in their curare experiments is a mere accentuation of the slighter rise obtained from similar stimulation in normal animals. They consider the possibility that the action on the vasoconstrictor may be a secondary result of primary psychic excitation, and reject it on the ground of the finding that the curare rise of pressure persists after cutting across the brain-stem. Dogiel (cited by Luciani, 12) found that normal animals showed vasoconstriction in response to auditory stimulation, and that the response disappeared under curare. We confirm the finding so far as normals are concerned. In the section of this paper dealing with reflexes in decerebrate animals we have reported our virtual failure to obtain vasoconstriction from either tactile or auditory stimulation in uncurarized decerebrate rabbits. We incline, therefore, to the view that both in our experiments and in those of Grützner and Heidenhain and of Dogiel the transient vasoconstriction seen in normal animals was due to a secondary influence on the vasomotor mechanism following psychic excitation. This interpretation places the reaction in precisely the same category with the familiar plethysmographic findings in man, wherein mental alertness is accompanied by arm shrinkage. In our opinion this view is strengthened by our observation that there is an apparent habituation to the stimulus. This is just what one would expect should follow repetition of a stimulus which depends for its effect on psychic excitation, particularly when the stimulation itself is of so mild a character as was used in this connection. The confinement of our positive results to the very early stages of urethane and ether anesthesia agrees also with this conception. By way of further confirmation we have the finding of Jacobson (14) that localized skin stimulation in human beings is unproductive of vasoconstriction through the operation of a direct reflex, although, as is well known, definite vasoconstriction occurs in man whenever mental alertness is elicited, however slight the exciting agent. The discussion up to this point summarizes into the generalization that mild tactile or auditory stimulation is productive of vasoconstriction only when it operates to bring about psychic excitation, which latter is the immediate cause of the vasomotor activity.

In addition to the transient vasoconstriction just discussed we obtained a similar, but more prolonged, reaction when an area of skin was chilled. The significant combination of influences here appears to be a physiologically intense stimulus (lowering the back temperature 8 degrees in two minutes) and a widespread area over which the stimulation is applied. That this combination is highly disturbing is shown by the prompt objection made by unanesthetized animals to an ice pack on the back. The cooling by pouring ether on the back was endured, but only, in our opinion, on account of fright induced by the odor of the ether. Deeply anesthetized and decerebrated animals gave the reaction regularly, showing that it is not dependent on psychic excitation.

Our most prominent exhibitions of vasodilatation occurred as the result of applying warmth to the skin, and of the two methods employed the more striking as well as the more constant was that which more nearly approached the normal conditions of the environment, namely, the gradual warming of a large area of the back by means of an electric light. There is nothing in our experiments to show whether this dilatation is reflex or is due to a direct effect of warming the blood, but the experiments of Winkler (cited by Bayliss, 10) gave evidence that a similar manifestation was definitely of reflex origin. A feature we wish to emphasize in connection with this reaction is that it occurs in response to a very gentle type of stimulation. If we may judge by the behavior of conscious animals, no disturbing element was contained in the back warming until it had been in operation for a number of minutes. Our other method of eliciting vasodilatation, by dipping a leg into water, gave much less uniform results. The sensory area involved was relatively limited, and the range of stimulation-strength which would evoke the reflex was narrow; cold water and water of moderate warmth gave negative results; water too hot aroused skeletal-muscle activities which nullified the observations. In several cases the response was reversed; this occurred usually, but not invariably, at the highest temperatures that could be used satisfactorily. The suggestion is that the attainment of water temperatures of definitely painful character tends toward the vasomotor response usually associated with pain, namely, vasoconstriction, while the gentler stimulation of warmth tends rather to induce vasodilatation. In none of our experiments with thermal stimuli was there any indication that psychic excitation is concerned in the reaction. The experiments of this group, while harmonizing on the whole with the accepted view that the specific effect of warmth stimulation is vasodilatation, and of cold vasoconstriction, seem to us also susceptible of explanation in terms of the volume of nervous discharge set up. That is, that gentle or restricted stimulation, evoking only a moderate discharge, induces typically vasodilatation, while intense or widespread stimulation, which sets up a considerable volume of nervous impulses, tends to arouse vasoconstriction. It will be seen that this is an attempt to avoid relating particular vasomotor reactions to the stimulation of specific sense organs, and to assign them rather to certain volumes of nervous discharge. In a subsequent communication additional evidence to this effect will be adduced, and a more detailed consideration of the bearing of this conception on the whole problem of reflex conduction presented.

SUMMARY

Experiments on the effects on ear volume of tactile, auditory and thermal stimulation in normal, decerebrate and narcotized rabbits lead to the conclusion that when vasoconstriction follows mild stimulation it is to be interpreted as a secondary effect, following primary psychic excitation. Gentle or limited stimulation not arousing mental alertness typically induces vasodilatation. Reflex vasoconstriction, not dependent on psychic excitation, requires intense or widespread stimulation for its elicitation.

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STUDIES IN PLACENTAL PERMEABILITY

I. The Differential Resistance to Certain Solutions Offered by the Placenta in the Cat

R. S. CUNNINGHAM

From the Anatomical Laboratory of the Johns Hopkins University

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The great development of chemistry in its relation to vital phenomena during the past twenty years has made imperative a more accurate and comprehensive examination of the various problems concerned with the permeability of the placenta. The many forces controlling the passage of substances into cells and through cellular membranes have become much better understood, and since the placenta can be considered as a cellular membrane separating the maternal circulation from the fetal, these advanced conceptions are available for application in the study of its permeability, so long recognized as of great importance in the physiology of the fetus. The greatest proportion of all workers in this field have approached the study of placental transmission by administering substances which might be qualitatively recognized in the fetus, and have merely stated whether or not they passed. Some have indicated the duration of the experiment, while others seemed to have considered the recognition of the substances as sufficient. Much of this work will, however, prove of permanent value because the large number of substances that have been used will permit of various classifications, and thus indicate further lines of investigation.

Nicloux (11) has made use of this opportunity, and has divided the substances into two groups, as follows: *a*, Substances soluble in water and diffusible, crystalloids; these traverse the placenta. *b*, Substances insoluble in water and not diffusible, colloids; these do not traverse the placenta. He has also (12) studied very accurately the transmission of ethyl alcohol administered to pregnant dogs and guinea pigs, and has found that in about one hour and thirty minutes after the introduction an equilibrium has been reached between the alcoholic content of the maternal and fetal bloods. Combining the results of his own experiments with his analysis of the work of others, he concludes that while many other factors should be considered simple dialysis is the most important.

As one considers the question of approach to a further study of this most extensive and complex subject, another classification seems advisable, in order to indicate the possible methods which may be utilized. All substances coming into contact with the placental membrane on the maternal side must fall into one of two great classes, those normally present in the circulation and those which reach it accidentally. It is obvious that the former will meet with an established mechanism which is adapted to furnish constantly some given type of activity, varying with the substance under consideration. On the other hand the large number of foreign substances which have been studied in one way or another, and the still larger number which we can easily conceive of obtaining adventitious entrance to the maternal circulation, must of necessity meet a very different condition because of the lack of any specific adaptation on the part of the membrane. Any study of foreign substances must be secondary to the search after the primordial reactions of the placenta to normal constituents of the maternal blood, but nevertheless these reactions may be often studied to advantage by methods which utilize such adventitious materials.

From the standpoint of the physical laws which substances obey in their relations to membranes they obviously fall into the following groups: gases, liquids, crystalloids and colloids. Nicloux (13), in reporting elaborate observations on carbon monoxide, concludes that this gas, as well as oxygen and carbon dioxide, follows exactly the laws of gaseous diffusion in traversing the placenta. His work on alcohol has been referred to; his studies on chloroform and ether are of equal interest and importance. In the study of crystalloids there has been no work comparable to Nicloux's on gases and liquids. Among the better contributions on the relation of colloids to the placenta are those of Wertheimer and Delezenne (19) on peptones, Goldman (8) on acid-azo-dyes, Ascoli (1) on albumens, and Wertheimer and Meyer (20) on methemaglobin.

It is evident from this brief and incomplete outline of the work that has been done that the point of optimum attack for further investigations must be in the study of fluid and salt interchange between the mother and fetus. The information obtained from a careful investigation of this mechanism, with any method, must be of great importance both directly and in the study of the more complex activities of the placenta in relation to the metabolism of proteins, fats and carbohydrates. The most fruitful method in the study of the normal pathways of fluid drainage is that proposed by Weed (17), and used by him to demonstrate the route followed by the cerebrospinal fluid in draining from the subarachnoidal spaces into the great sinuses. He injected a solution containing 0.5 per cent each of potassium ferrocyanide and iron ammonium citrate into the subarachnoidal spaces, and precipitated the Prussian blue in the course of its drainage through the arachnoidal villi. Later Shipley and Cunningham (16) demonstrated the passage of fluid into the veins and capillaries of the omentum by withdrawing that structure from the peritoneal cavity through a midline incision, and immersing it in a similar solution. Many other investigators have made use of the Prussian blue reaction, but have not used the balanced solutions for the study of fluid pathways. It is essential that these solutions be practically balanced to avoid the formation of soluble Prussian blue during the process of fixation. Both these salts pass readily through blood vessel walls, through arachnoidal villi, through pectinate villi and through the kidney. In each of these cases it is probable, if not certain, that they follow the normal direction of fluid passage.

When projected, the study of these salts in their relation to placental permeability was intended to analyze, if possible, any differential adaptation of parts of that structure to the passage of water and salts. In the case of these salts we are dealing with substances which are foreign to the organism, but which offer the advantage of being easily followed, not only to their ultimate location, but in the route traversed. A study of the transmission of salts normally present in the maternal organism would be of more value if they could only be followed in their course through the placenta. If by this method any information can be gained regarding salt and fluid interchange, this may be applied later to considerations concerning the normal salt constituents of the blood. Starting with the assumption that fluids pass easily from mother to fetus it was hoped that the cellular mediation could be analyzed in the same way as in the cerebrospinal system, the eye and in the peritoneum. It was a priori thought that both sodium ferrocyanide and iron ammonium citrate would easily traverse the placenta, because comparable salts had been shown to do so with ease by previous workers. Bar (2) described experiments in which potassium ferrocyanide was injected into the uterine veins of rabbits, and was found after thirty minutes in the amniotic fluid. If then the potassium salt passes with such ease, it seemed probable that the resistance would not be greater to the less toxic sodium ferrocyanide. No such comparison was possible in the case of the iron ammonium citrate. However, certain organic salts have been found to traverse the placenta; Porak (14) showed that potassium acetate passed in experiments on rabbits, Zweifel (21) used sodium salicylate, administered to women in labor, Fehling (7) used sodium salicylate on rabbits, Launois and Briau (10) used this same substance on guinea pigs and rabbits, and Savory (15) used strychnine acetate on cats, dogs and rabbits. It is only by observing the effects of many different salts that we may determine any of the laws governing the actual interrelation of salts and tissues. In the placenta it is well known that the acid-azo-dyes and colloidal metals, as well as many other colloidal sols will not pass from the maternal to the fetal circulations. From this it is clear that there is a definite resistance offered to a group of substances whose molecules are large but which have, as far as we know at present, no other characteristic in common. These substances will all pass through capillary walls, through the lining of the peritoneum and through the kidney; and they will diffuse into certain cells in the body while other cells remain impermeable to them. On the other hand, capillary endothelium appears to be relatively impermeable to proteins, sometimes completely so, while easily permeable to the substances named above. Relative permeability is then a certainty both in tissue cells and in those cells functioning as membranes separating body fluids.

The experimental animals used in the past for these investigations on placental permeability have been confined almost entirely to rats, guinea pigs and rabbits. A very few workers have used dogs, cats and sheep; while many have performed experiments on women in labor, but few comparative studies have been made using the same substance in different species of animals. Bremer (5) calls attention to this in discussing anatomical characteristics of various placentae, and suggests that many of the conflicting statements in the literature can be explained by the variations in the animals used, and that more progress could be made by using a greater variety of species in the study of any given substance. He has studied the placentae in a large group of animals, in relation to the problem of fetal excretion, and therefore in relation to other organs which may assume all, or at least part, of the excretory function: the pronephros, the Wolffian body and the kidney. He says:

> Mammalian embryos may be divided into two classes; those which retain functional Wolffian bodies until the kidneys are sufficiently developed to excrete urine, as is the case in birds and reptiles, and those in which the Wolffian bodies degenerate before the kidneys reach functional ability. The first class includes the pig, sheep and cat; the second the rabbit, guinea pig, man and rat. . . . . In those animals without the possibility of a continuous urinary excretion within the embryo, i.e., with an early degeneration of the Wolffian body, the placenta is provided with an apparatus similar to that found in the glomeruli of the Wolffian body or the kidney, thin plates of epithelium overlying the fetal capillaries. These appear in the placenta at about the time when the Wolffian body commences to degenerate, or in the case of the rat, which never develops mesonephric glomeruli, at about the time of the normal development of the glomeruli in other embryos. These plates continue and increase in number till term. They are apparently of greater extent in animals whose embryos are provided with large Wolffian bodies. In the placentae of those animals with a continuous embryonic urinary excretion, similar plates are not found, whether the placentae be of the opposed or conjoined type. From these facts it appears that embryonic and fetal urinary excretion takes place wholly through the placenta in the rat, at first through the Wolffian body and later through the placenta in the rabbit, guinea pig and man, but never through the placenta in the pig, sheep or cat. A knowledge of these differences should lead to more intelligent experiments on the permeability of the placenta.

Bremer does not discuss the question of differential permeability, but his observations are extremely interesting and important from a physiological point of view, and particularly emphasize the absolute necessity of studying all problems of embryological physiology from a comparative viewpoint, as variation in species may be sufficient to produce very conflicting results. Bremer's theory, founded entirely upon anatomical observations, has received but very little real physiological examination; it is, however, supported by the previous results obtained by Krunkenberg (9) who found that substances which did not pass through the placenta in dogs and cats, but were retained by the fetus, found their way easily into the maternal circulation in the case of guinea pigs and rabbits. Savory (15), on the other hand, described experiments which are contradictory. He found that strychnine acetate when introduced into canine fetuses caused convulsions in the mother in nine minutes and death in twenty-eight. His experiments on cats and rabbits gave the same results. Bremer considers: ". . . . that these modified plates have apparently become inactive membranes, through which a purely physical osmosis may take place, but which themselves may be supposed to be physiologically inert." While he applies his observations to excretion only, there seems to be no reason to preclude that the permeability is equal in both directions for any membrane which is physiologically inert. Therefore experiments dealing with maternal to fetal transmission in animals having plates must be considered equivalent to a similar experiment in the opposite direction.

Goldman (8) has shown that trypan blue will not pass the placental barrier in the rat, but is easily excreted by the maternal kidneys. In some unpublished experiments I have found that trypan blue introduced into the circulation of rabbit fetuses in a late stage of gestation, was excreted by the fetal kidneys, but did not pass through the placenta into the maternal circulation. These experiments have also been performed on the fetuses of cats with the same results. It is obvious at once that the plates of the renal glomerulus must be different in their permeability from those in the placenta, when measured by this one class of colloidal sols. This does not in any way invalidate Bremer's conclusions that these placental plates indicate the route of fetal excretion and are physiologically inert, but prevents accepting the plates found in the placenta as an exact equivalent of those in the kidney.

Admitting Bremer's hypothesis to be substantiated, only one phase of the general problem will be settled because even if certain species of animals have specialized membranes which permit the passage of substances excreted by the fetus, still there is the essential necessity of the fetus receiving salts, water and foodstuffs from the mother, and these must pass the placenta irrespective of any variation in species. The problems still unanswered regarding the permeability of the placenta are innumerable. They are of vital interest both as regards their local and direct significance and in their bearing on phenomena elsewhere in the organism. They range from the general problem of cell permeability and the resistance of membranes, to special ability of the placenta as an organ to select, change and even synthesize substances needed in the fetal metabolism. The placenta is a single structure which probably exercises the combined functions of many types of cells, and therefore any laws which may be found governing its activities will undoubtedly be applicable in part to other cells. But the placenta differs so essentially in function from any other single tissue that even those laws having the most widespread application will probably have special significance here. As far as is possible all the laws developed for single cells and simple membranes should be applied to the special case of this complex and more primitive structure.

The present communication is the first of a series projected, and partly under way, having as their object the analysis of some of the more fundamental problems relating to placental permeability.

The plan has been to first analyze, by whatever methods are available, the normal fluid and salt interchange and to determine if possible what forces are most active in these processes. Then to determine as far as possible the nature of the placenta as a membrane and by what means certain groups of substances are excluded by it, while others are allowed to pass; and to ascertain whether this reaction is comparable to others in the body or peculiar to the placenta alone. Finally it is desired to determine what rôle the placenta plays in the fetal metabolism, and to establish the nature of its relation to each type of substance that must be recognized as essential for the fetal nutrition.

MATERIALS AND METHODS

In this attempt to analyze the fluid interchange cats were used exclusively, and those in a late period of gestation were selected. The entire series consisted of animals that had passed the fortieth day of pregnancy. The youngest fetus in the series measured 78 mm., and the oldest 121 mm., crown rump diameter. The technique has been uniform throughout except in certain experiments designed expressly to test some specific point arising in the course of the work. All animals received balanced solutions of sodium ferrocyanide and iron ammonium citrate, the usual strength being 1½ per cent of each salt. The percentage strength was varied in some cases; these will be mentioned in the appropriate place. In all operations the solution was administered as an injection by means of a burette attached to a glass cannula introduced into the vein of the forearm. Intratracheal ether anesthesia was used in the majority of these experiments, and was found entirely satisfactory. It is well known that the cat maintains almost normal respiratory and circulatory functions under ether anesthesia. After establishing results which were entirely constant for ether, controls were decerebrated following Weed's (17) technique, and certain crucial experiments repeated. The results obtained were in all cases exactly the same as found in similar experiments under anesthesia. By this the possibility of any effect by the anesthetic on the placenta was eliminated, and this was essential since we know from Nicloux's (11) work that ether passes the placenta and it might in consequence render the placental membrane either more or less permeable to the substances used in the experiment. The salts were weighed out and dissolved immediately before the experiment. They were not mixed until the cannula had been inserted, when they were poured together, and then directly into a burette and the injection begun. Both sodium ferrocyanide and iron ammonium citrate are eliminated very rapidly by the kidneys, so that some method had to be devised in order to keep the concentration of the salts constant in the blood stream. The method finally decided upon was the preliminary interruption of the renal excretion by ligation of the renal vessels. After this the desired amount of the experimental solution was run in slowly, and the animals kept undisturbed until sacrificed. In order to avoid the possibility of this procedure causing changes in blood pressure, experiments were performed to test the effect of administering these salts after ligation of renal vessels. Amounts more than double those usually given caused only a transient rise of pressure, so that the hydrostatic element may be ruled out. This method proved so satisfactory that it was used entirely, except for a few animals in which a continuous injection was maintained throughout the entire experiment of just enough solution of the salts to keep them present in the blood stream. Quantitative determinations were not made, but precipitation tests of Prussian blue in the maternal blood plasma were carried out in order to indicate the presence of both salts in approximately the same amounts, as in experiments done with the preliminary renal ligation. These experiments were considered necessary to exclude the possibility of the lack of excretion from the kidneys having an effect upon the placenta, but the results with the kidneys functioning and when ligated were the same. Cats were exposed to varying doses for periods of time ranging from ten minutes to ten hours, and were then killed. The uterus was opened so that the amniotic sac bulged out, the fluid carefully withdrawn with syringe and needle to avoid contamination, and then examined chemically. The fetuses were removed and their abdominal cavities opened; whenever the bladder was distended it was treated in precisely the same manner as the amniotic sac. Small blocks of fetal kidney and placenta were fixed directly in Bouin's fluid, the acetic acid in this mixture being sufficient to precipitate the Prussian blue. In the analysis of the fluids under examination, solutions of ferric chloride and ferric sulphate were used to determine the presence of sodium ferrocyanide, while a ferrocyanide was used to test for the ferric iron. It was found that there is sufficient iron present normally in fetal urine and amniotic fluid to yield a Prussian blue reaction with sodium ferrocyanide and hydrochloric acid when allowed to stand; the test for ferric iron was therefore read immediately. The exact details of all tests will be given when the reports of the histological findings are made, and need not be considered further here. The results of the introduction of these salts into the blood stream of the living fetus are from a series of experiments, still quite incomplete, concerning the general methods of fetal excretion, and which will be reported later. Such materials as bear upon the present problem will be referred to here. In regard to the question of the validity of the tests given above the following experiment will indicate both the delicacy of the test and the certainty of finding our reagents in the fetal urine or amniotic fluid if they had reached the fetal circulation.

Cat P. P. 23. Intratracheal anesthesia. Midline abdominal incision with exposure of uterus.[1] One cubic centimeter of a solution containing 1 per cent each of sodium ferrocyanide and iron ammonium citrate was introduced into the umbilical vein of one of the fetuses, which was removed after two and a half minutes, and the bladder exposed. A drop of acid added to the fetal urine gave a brilliant blue color. Another fetus was exposed and the same procedure followed except that the time interval in this case was twenty minutes. The bladder was found to be contracted, test of the amniotic fluid yielded Prussian blue. The fetuses measured 80 mm.

It is quite evident that the fetal kidney will very quickly eliminate both salts, and that if the bladder has contracted after this elimination has begun the amniotic fluid will show their presence. We found, in many of our experiments on the fetus, that the bladders were empty. From this it seems probable that the increased flow of urine had stimulated them to contract. It is evident that the tests used were sufficiently delicate to indicate any exchange of these salts between mother and fetus even when relatively small doses were given to the mother intravenously.

EXPERIMENTAL RESULTS

The results of many experiments indicated that the placenta of the cat can be functionally divided into three parts in regard to its reaction to the salts here used. Duval (6), in studying the histology of the placenta, has described three layers in the labyrinth of the cat during later states of gestation: the maternal endothelium which remains intact and is included within the invading trophoblast of the ovum;

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[1] The technique of these operations will be reported with the other details on embryonic excretion.

the ectodermal layer, which is syncitial in character; and the embryonic endothelium which lines the embryonic vessels even where they penetrate deeply into the syncytial ectodermal layer. The maternal endothelium is everywhere continuous, the cells being very prominent in contrast to the thin, delicate cells of the fetal capillaries. In every experiment of the entire series the reaction of the maternal endothelium was precisely the same. No variation in this reaction could be discovered in any part of the placenta, despite the fact that certain areas showed more blue than others on examination with the low power of the microscope. This was probably attributable to a variation in the blood supply due to mechanical causes. Everywhere on histological examination the characteristic blue granules could be seen both in the bodies of the endothelial cells and lying between them and the ectodermal layer. In exactly the same manner the fetal endothelium is easily permeable to both salts. This passage takes place very rapidly because in experiments of ten minutes duration, the blue granules are found in, and beyond, the endothelial cells. It is impossible to deny that the salts may pass between the endothelial cells as well as through them, although there are no granules precipitated in a manner which would indicate this, as they seem to be equally distributed throughout the entire extent of the cytoplasm. That they both pass through the maternal endothelium rapidly is evident and that they pass only through, and never between these cells is probable, but can not be stated as absolutely proved. This difficulty is obviously met with in any membrane, and has been commented upon by Weed (17) in regard to the arachnoidal villi; this will be referred to later. It is probable then that the maternal endothelium present in the placenta has the same degree of permeability in regard to these salts as endothelium elsewhere in the body. At least it can be asserted that they pass here in the same manner, that is, with the same histological distribution as was shown in the veins and capillaries of the omentum by Shipley and Cunningham (16). The maternal endothelium is easily permeable to both of these salts, the fetal endothelium is equally so, but the fetal ectoderm reacts in an entirely different manner. The behavior of the ectodermal layer permits the division of the experiments into two groups, relative both in terms of permeability and duration of time. In the first group are included experiments of duration varying from ten minutes to four hours and a half. In not one of these has any trace of either sodium ferrocyanide or iron ammonium citrate been demonstrated in amniotic fluid, fetal urine, or tissue extract. And in every case the bladders of the fetuses were found on removal to be full of urine. In the second are included those from five to ten hours in duration, the latter being the maximum of the series. In all the experiments in this group traces of sodium ferrocyanide were found in the fetal urine or amniotic fluid, but in none of them could even the slightest trace of the iron ammonium citrate be demonstrated. The amount of ferrocyanide which had passed the placenta was in no case very large. In experiments of from five to six hours only a trace was found, this was increased somewhat in those of longer duration, the maximum being approximately that amount which would be excreted by a fetus in twenty minutes if ½ cc. of a 1 per cent solution had been injected intravenously. In marked contrast to the fetuses of the first group, the bladders were found in most cases to be contracted and entirely emptied of their urine. And in every one of these the ferrocyanide could be demonstrated in the amniotic fluid. This interesting reaction of the fetal bladder is illustrated in the following experiments:

Cat P. P. 25. 9:00 a.m. Intratracheal ether.

Each kidney delivered retroperitoneally, and the vessels ligated.

Cannula inserted into vein of left forearm of mother, connected to burette containing 50 cc. of solution of 1½ per cent each of sodium ferrocyanide and iron ammonium citrate.

9:20 a.m. Intravenous injection begun.
9:30 a.m. 5 cc. in.
9:40 a.m. 10 cc. in.
9:50 a.m. 15 cc. in.
10:00 a.m. 20 cc. in.
10:10 a.m. 25 cc. in. Injection stopped.

Animal in excellent condition. No change after injection began except increased salivation. The animal remained in excellent condition until sacrificed at 3 p.m. Total duration of experiment, five hours and forty minutes. The uterus was immediately removed and found to contain three fetuses in the left horn and two in the right. The uterine wall was incised over the caudal end of each fetus in such a manner that the amniotic sac bulged out freely; this was punctured with a needle and the fluid withdrawn. Each fetus was removed, all were noted to be alive. The abdominal walls were incised and the urine removed when present.

Right horn of uterus: Both fetuses had empty bladders, the amniotic fluids contained a trace of ferrocyanide, but no iron ammonium citrate. They measured 89 mm. and 92 mm.

Left horn of uterus: Fetus 1. Bladder full, but not distended. Urine negative for ferrocyanide and iron ammonium citrate. Measured 91 mm.

Fetus 2. Bladder full, and distended. Traces of ferrocyanide in urine, no iron ammonium citrate. Measured 90 mm.

Fetus 3. Bladder empty, trace of ferrocyanide in amniotic fluid; no iron ammonium citrate. Measured 94 mm.

Cat P. P. 28. 9:30 a.m. Intratracheal ether.

Each kidney delivered retroperitoneally and the vessels ligated.

Cannula inserted into vein of left forearm of mother, and connected to burette containing 50 cc. of solution of 2 per cent each sodium ferrocyanide and iron ammonium citrate.

9:50 a.m. Intravenous injection begun.
10:00 a.m. 5 cc. in.
10:10 a.m. 10 cc. in.
10:20 a.m. 15 cc. in.
10:30 a.m. 20 cc. in.
10:40 a.m. 25 cc. in. Injection stopped.

The only immediate effect of injection was increased salivation. Heart rate remained about same throughout.

6:20 p.m. 10 cc. of blood withdrawn from maternal heart, centrifuged immediately, a few drops of acid added, blue color developed.

6:20 p.m. Ether to death.

Total duration of experiment, eight hours and thirty-five minutes. The uterus was removed and found to contain four fetuses, two in each horn. The uterine wall of each fetus was incised carefully and the amniotic fluid removed and tested. The fetuses removed and their bladders examined. In three, the bladders were found to be entirely empty, and the amniotic fluid was positive for ferrocyanide and negative for iron ammonium citrate. In the fourth fetus the bladder contained a single drop of urine which gave a positive test for ferrocyanide with ferric chloride and the amniotic fluid reacted as in the other three fetuses. Tests for iron ammonium citrate were negative. The fetuses were all alive and measured 80, 82, 84 and 86 mm. respectively.

On histological examination of the placentae of animals subjected to experiments of this type, the reaction of the ectoderm is most remarkable. In the shortest experiments, of duration less than an hour, the Prussian blue is found throughout the maternal endothelium and in a fine blue line between this layer and the syncytial ectoderm. There are no granules to be seen anywhere within the ectoderm, but they are closely adherent to the outer border both of the syncytium and the inter-capillary giant cells. Indeed these present a most remarkable picture of large round cells everywhere surrounded by a fine blue border, but nowhere can any granules be seen within their cytoplasm. In experiments of longer duration, about two hours, blue granules are to be seen within the ectodermal layer; these have not penetrated far into the cytoplasm but tend to form lines relatively close to the maternal poles of the ectodermal nuclei. This arrangement is very irregular, frequently the granules collect in small masses instead of lines and filaments. In experiments of six, eight and ten hours duration there is very little change in the histological findings. The blue granules are found deeper in the cytoplasm of the ectoderm, in some places they are scattered almost throughout the entire structure. None can be seen in the fetal endothelium, or between the endothelial cells and ectoderm. Also the inter-capillary giant cells have absorbed a small amount of the two salts so that a few fine blue granules are seen in the peripheral third of the cytoplasm, while the rest of the cell is entirely clear.

The principal advantage of the method used in these experiments is that, by the precipitation of Prussian blue in situ, it is possible to interrelate the results obtained by examination of fetal excretion with the histological location of the point at which these results must have been determined. These placentae are sufficienty interesting to merit a more detailed histological description than would be advisable here; this will be given later when comparative results on other species are available.

DISCUSSION

The principal facts demonstrated by these experiments are the differences in the permeability of the endothelial and ectodermal layers of the placenta, and the differences in the resistance offered to the two experimental salts by the fetal ectoderm. It is evident that the placenta may be considered as two protoplasmic membranes which are directly applied to each other in such a way that they appear anatomically as a single membrane separating the two bloods, but have in reality entirely different physiological properties.

In considering a given living membrane we have to deal with three phases of activity, the entrance of a substance into the membrane, its passage through the membrane, and its exit from the membrane. The factors involved in each of these may be entirely different, and those relating to one membrane may differ entirely from those relative to another. The simplest membrane in the organism is such a one as that described by Weed (17) in the arachnoidal villus where he found the exchange through the membrane to be effected by a simple hydrostatic filtration; osmotic pressure having little or no participation. That the passage of sodium ferrocyanide through the entire placenta is not a simple filtration dependent upon hydrostatic pressure alone, is evident from several experiments carried out expressly to determine this point. After animals had been exposed in the usual manner and to the usual dose of this salt, the blood pressure was raised to a maximal degree by the continued introduction of physiological saline under pressure of about 500 mm. of water. Despite this procedure no variation in the passage of this salt as previously determined in animals with normal blood pressure was noted. Simple filtration by hydrostatic pressure can therefore be considered as a negligible factor.

It is evident that all materials necessary for the nutrition of cells and all products of cell metabolism that either come from the blood stream or are eliminated by it, must pass through the capillary wall. Therefore the endothelial membrane must be permeable to water, salts and even to some colloids, since some of the substances included above are in that state. This remarkable degree of permeability shown by capillary endothelium has been explained in various ways. It has been suggested that the endothelial cells are capable of separating from each other and allowing large molecules, as well as small, to pass through by simple filtration. Others have thought that the intercellular cement substance is physiologically inert and contains large pores through which diffusion of colloids may take place. The other possibility is, that the endothelial cells are constructed in such a way that their cytoplasm is permeable to large molecules. The nature of the mechanism by which permeability is regulated still remains unsettled; Bayliss (4) after reviewing many observations at variance with each other concludes that:

> Different views are held as to that property of a membrane which makes it permeable to some solutes and impermeable to others. Reasons are given in the text for accepting, with some modifications, the original sieve theory of Traube, according to which the passage of a solute through a particular membrane, depends on the size of the pores in the membrane in relation to the molecular, or particulate, dimensions of the solute. The hydration of solutes must be taken into account. In a few cases, the question of solubility in the substance of the membrane appears to play a part.

That capillary endothelium is permeable to both iron ammonium citrate and sodium ferrocyanide has been shown in the case of the omentum by Shipley and Cunningham (16). The forces involved in the passage of these salts through the maternal endothelium of the placenta are probably no different from those operative in the case of any other capillary wall. That osmosis and diffusion are the most important is assured, but that other factors are involved is shown by the observations that substances can be absorbed from the peritoneal cavity against osmotic pressure and concentration gradient, and likewise substances can be secreted by the kidney under similar circumstance. While these factors are not yet entirely understood they are explained in terms of negative osmose, which in turn is explained in terms of electro-endosmose, or the pull of a charged particle through a membrane pore by an opposite charge on the membrane (Bartell and Hocker (3)). The experiments in this series do not assist in analysing the factors pertaining to the permeability of the maternal endothelium, and therefore of endothelium in general, except that they show conclusively the passage of these salts through the cytoplasm of the cells.

We have seen that both sodium ferrocyanide and iron ammonium citrate penetrate the ectodermal layer in about two hours, while the majority of cells in the mammalian organism appear to be semi-permeable as regards these salts, even when exposed to them for much greater periods of time. So that the limiting membrane of the fetal ectoderm must differ in its normal permeability from the limiting membrane of cells in general. The differences in structure or composition between membranes permeable to certain crystalloids and others semi-permeable to them, are as yet unknown. Whether other factors than osmosis and diffusion are concerned in the penetration of the ectodermal membrane can not now be stated. When we consider the passage of these substances through the cytoplasm and the exit of one while the other is retained, other factors must be considered. After about five hours the sodium ferrocyanide passes through the cell membrane adjacent to the fetal capillaries and can be detected very quickly in the fetal urine. At this time tests fail to reveal any citrate as having passed through, nor do experiments of several hours longer duration yield different results. It is evident, therefore, if these tests are sufficiently accurate, that no citrate has been able to pass out of the fetal ectoderm despite the fact that it has unquestionably entered it. The rate at which sodium ferrocyanide passes through the placenta is extremely slow in comparison with its passage through other living membranes which have been studied. While this does not prove, it certainly suggests that other factors than osmotic pressure and diffusion are involved. It is possible however, to conceive that the ectoderm is so difficultly permeable to sodium ferrocyanide that we are actually measuring the normal time element of its diffusion, in other words, the ratio of osmotic pressure to the resistance of membrane. The determination of the osmotic pressure of the maternal and fetal bloods would be of great assistance in indicating how much salt exchange depends on this factor. Such determinations have not been done. It is evident that if an osmotic balance is maintained this exchange would be easier than if the presence of metabolic products in the fetus should have raised the pressure on that side; but it is possible to conceive that even if the osmotic pressure in the fetal blood is equal to, or lower than in the maternal blood, the retardation of the passage of sodium ferrocyanide might be caused by an unstable combination with intracellular compounds; on the other hand, if the fetal pressure be proved to be higher, some activity comparable to that of secretion must be postulated.

We can conclude much more surely that other factors than osmosis and diffusion are operative in regard to the peculiar action of the ectodermal membrane toward the iron ammonium citrate, because both the ferrocyanide and citrate are known to diffuse through many different membranes at approximately equal rates. Of course it must be admitted that we have not excluded the possibility of the citrate passing through the ectodermal layer if exposed for a longer period of time. In this case we would be forced to conclude either that some intracellular activity had delayed the citrate longer than the ferrocyanide, or that the fetal surface of the ectoderm was much more easily permeable to ferrocyanide than to the citrate, and consequently different, not only from many other living membranes, but also from the maternal side of the ectoderm. On the other hand, if longer experiments substantiate those of six, eight and ten hours duration, we are forced to the conclusion of some even more radical change taking place within the fetal ectodermal layer. Two possibilities may be mentioned, although it is evident that both are entirely without experimental evidence. First, it is conceivable that some substance present in the cell prevents the further passage of the citrate by a specific adsorption. Second, as many organic acids and salts of organic acids are changed and broken down in the metabolism of the animal body, it is therefore quite possible that the ectodermal syncytium is capable of breaking down iron ammonium citrate into substances no longer detectable by our tests, which were after all only capable of demonstrating the presence of ferric iron. It should certainly prove feasible to devise methods for determining whether this salt, and perhaps many other salts of organic acids, can be decomposed in the fetal ectoderm. We have considered so far, in endeavoring to explain the results of our experiments, the effects that the tissues involved may have had upon the salts introduced; but it is necessary to consider also what effect the salts may have had upon the tissues. While the death of the cell after exposure to these salts can be easily determined by the brilliant blue staining of the nucleus, minor injuries do not produce any change which can be recognized in histological preparations. In general it is known that permeability of cells is increased by injury; if then the ectoderm has been changed by these salts we must conclude that it is normally semi-permeable to them. But it is extremely difficult to understand how one of these salts can be retained and the other pass out into the fetal blood, if they can not penetrate the ectoderm until they have injured it in some degree. Admitting that it is injured, the results which have been obtained can only be explained in one of two ways: either the injury is insufficient to destroy the activity which prevents the passage of the citrate, or else this function is a chemical reaction developed as the result of the injury. These explanations are both rather improbable, so that it seems more likely that the degree of injury is slight. Whether or not longer exposure to these salts, and particularly to the citrate, would depress the cell activities enough to allow the citrate to pass through is entirely problematical and need only be stated here as a possibility. Finally, it is worth while to consider what bearing these experiments have upon the theory advanced by Bremer and referred to before. According to his theory excretory products of fetal metabolism in the cat do not pass through the placenta, but are excreted by the fetal pronephros, Wolffian body or kidney, as the case may be. Since the fetal as well as the adult kidney excrete both of these salts very rapidly, i.e., within two or three minutes, and since it requires five hours for one of them to pass the placenta, it seems strong evidence that his interpretation of the absence of placental excretion in the cat is correct. Although still quite incomplete, the work on the excretion of the embryo, referred to above, also substantiates Bremer's view as far as the cat is concerned.

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THE PRODUCTION OF INTRACELLULAR ACIDITY BY NEUTRAL AND ALKALINE SOLUTIONS CONTAINING CARBON DIOXIDE

M. H. JACOBS

From the Laboratory of Zoölogy, University of Pennsylvania

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It has recently been pointed out by the author (1) that the apparently contradictory views as to the mode of action of CO_2 on the mammalian respiratory center expressed on the one hand by Winterstein (2), Hasselbalch and Lundsgaard (3), (4) and others, and on the other by Laqueur and Verzár (5), Hooker, Wilson and Connet (6) and Scott (7) are not necessarily in conflict. Carbonic acid may conceivably, like any other acid, act primarily through its hydrogen ions, as the first group of workers have held, and yet differ so greatly from other acids in certain peculiarities connected with its powers of penetrating living cells as to have practically as specific an action as that postulated by the second group.

One of the most interesting of these peculiarities is the relatively small importance of the hydrogen ion concentration of a solution containing carbon dioxide in determining the degree of intracellular acidity produced in a cell exposed to it. The author has already called attention to the fact that a neutral or even a slightly alkaline CO_2-bicarbonate mixture is practically as toxic to tadpoles as a pure CO_2 solution of the same concentration, and that such a solution has a sour taste. He has interpreted these results as being due to the fact that the H_2CO_3, whose dissociation is held in check by the bicarbonate, can freely enter cells (perhaps as CO_2), while the bicarbonate cannot, the carbonic acid dissociating when within the cells to the extent permitted by the new conditions of equilibrium prevailing there, which may easily result in a hydrogen ion concentration higher than that of the surrounding medium.

While the observations already recorded have probably been correctly interpreted, it has seemed desirable to supplement them with a case where the rise in intracellular acidity under the conditions in question is actually visible to the eye. Such a case might be furnished by a cell containing a sufficiently sensitive natural indicator whose color changes would give visible evidence of any increase in the hydrogen ion concentration within the cell. After a rather lengthy search, material of this character has been found in the colored flowers of *Symphytum peregrinum*, a cultivated plant belonging to the family *Boraginaceae*. These flowers, like those of many other members of the family, are pink in the bud, later becoming blue; the former color is associated with a higher, the latter with a lower hydrogen ion concentration. The exact pH of the turning point has not been determined, but is within the range of carbonic acid solutions.

The use of colored flowers to study cell penetration by acids, though Haas (8) obtained good results with this method, has not been very much favored in the past because of the fact that such cells are generally cuticularized and are not readily wetted by aqueous solutions. In the present case, however, it was found that if the end of the tubular corolla were snipped off with a pair of scissors and the whole flower were then slipped over the tapering end of a glass rod which could be dropped into a test-tube containing the solution to be studied, the change in color appeared quickly and regularly and could easily be followed, especially along the "ribs" correspondng to the attachment of the stamens, and at and above the depressions which are found at the points of attachment of the "corolla scales." To observe the change in color, a hand lens was used, though even with the naked eye the results were easily visible. For example, the difference between a flower exposed to a saturated solution of CO_2 and one kept in distilled water was usually apparent in five minutes at a distance of five or six feet. The change in color with weak solutions of acids always began on the "ribs" and above the depressions mentioned, and gradually spread from them to other parts of the flower.

As a considerable number of similar experiments with this material all gave essentially the same results, it will be sufficient to describe a typical one. Four flowers from the same plant, as nearly alike in color as possible, were selected. The first was exposed to a saturated solution of CO_2 in distilled water (pH approximately 3.8); the second to pure distilled water of pH between 5.0 and 6.0 (the slight acidity being due to CO_2 absorbed from the air); the third to an M/2 solution of $NaHCO_3$ saturated with CO_2 at atmospheric pressure and having a pH of *ca.* 7.4, and the fourth to an M/2 solution of $NaHCO_3$

considerably more alkaline than pH 8.0. The course of the experiment may be indicated most clearly in tabular form as shown in table 1.

It will be observed that the increase in acidity of the cells was not proportional to the pH of the external medium, since those exposed to the slightly akaline CO_2-bicarbonate mixture became distinctly acid, while those in the distilled water with a hydrogen ion concentration perhaps one hundred times as great did not. There was also comparatively little difference (up to the time when the bicarbonate finally

TABLE 1

Color changes in flowers of Symphytum exposed to various solutions

TIME	(1) DISTILLED WATER + CO_2	(2) DISTILLED WATER	(3) M/2 $NaHCO_3$ + CO_2	(4) M/2 $NaHCO_3$
11:20*	Blue	Blue	Blue	Blue
11:21	Pink color appearing on lower "ribs" and above depressions	No change	Pink color appearing on lower "ribs" and above depressions	No change
11:23	Upper "ribs" becoming pink	No change	Upper "ribs" becoming pink	No change
11:25	Pink spreading laterally from lower "ribs"	No change	Pink spreading laterally from lower "ribs"	No change
11:27	Lower portion of flower mostly violet with pink "ribs"	No change	Lower portion of flower mostly violet with pink "ribs"	No change
11:31	Upper portion of flower shows considerable pink and violet outside of "ribs"	No change	Upper portion of flower shows less violet than (1) but "ribs" are, if anything, pinker	No change
11:38	About the same	No change	About the same	No change
11:45	Same	No change	Same	Becoming slightly bluer than (2)
1:05	Same	No change	Slightly bluer than before but still pinker than (2)	Beginning to turn greenish
2:30	Same	No change	About the same as (2)	Decidedly greenish

* Started.

began to penetrate) between the two solutions saturated with CO_2, though the concentration of hydrogen ions in the one was approximately four thousand times that in the other. Evidently, therefore, the neutrality or slight alkalinity of a solution does not rule out the possibility of decided effects of hydrogen ions where CO_2 is one of the substances concerned, and the stimulation of the mammalian respiratory center in Scott's (7) experiments by blood of pH 7.6 would not necessarily be in conflict with the orthodox view of the rôle of hydrogen ions in this process.

It may perhaps be of interest to show that the effects of CO_2 on living cells here described may be imitated by a simple model whose construction depends on the fact that CO_2 is freely soluble in xylene (as well as in other lipoid solvents and lipoids) while $NaHCO_3$ is not. The "cell" is made as follows. A small "shell vial," 10 by 35 mm., is filled to within 4 mm. of the top with a solution of phenolsulphonephthalein made very slightly alkaline by the addition of a trace of $NaHCO_3$. It is next filled level full with xylene (from which the CO_2, if necessary, has been removed by allowing it to stand in contact with an aqueous $Ba(OH)_2$ solution, and the escape of the xylene is then prevented by the following simple expedient. A test tube is dipped into a fairly thick celloidin solution and the film which closes its mouth on removal is immediately pressed over the opening of the vial to which it adheres as a thin transparent covering, freely permeable to salts, acids, etc., but preventing the loss of the xylene when the "cell" is immersed in water. The vials as thus prepared are kept until needed in an upright position in water. In some cases cottonseed oil was used in place of xylene with essentially similar results, though it is less convenient to work with, and results are obtained more slowly with it than with xylene.

A typical experiment with "artificial cells" was the following. The solutions used were the same as those studied with the *Symphytum* flowers, namely, distilled water saturated with CO_2, distilled water, M/2 $NaHCO_3$ saturated with CO_2, and M/2 $NaHCO_3$. With the distilled water and $NaHCO_3$, there was no change in the color of the indicator; with the two solutions containing CO_2, though, as before, the actual hydrogen ion concentration of the one was perhaps four thousand times as great as that of the other, there was an approximately equal rate of change in color of the red indicator solution to a bright yellow. In both cases, the change began to be visible within two minutes and was complete in about an hour and a half. The line of demarcation between the red and the yellow portions of the solution was very sharp, especially at first.

The result of one alkaline solution turning acid when placed in another alkaline solution is a rather striking one. In this case it evidently depends on the relative solubilities of CO_2 (and perhaps H_2CO_3), on the one hand, and of $NaHCO_3$ on the other, in lipoids and lipoid solvents. In living cells there is frequently a close correlation between the lipoid solubility of substances and their powers of penetration. Without at all postulating a lipoid membrane in Overton's original sense, it is nevertheless possible that the relative solubilities of the substances concerned in the lipoid and aqueous phases encountered in the structure of a typical cell may have much to do with the physiological behavior of CO_2-bicarbonate mixtures.

In connection with the points already mentioned, it is of interest to compare the penetrating powers of CO_2 with those of other acids, particularly those which, from the results of previous workers, appear to enter living cells with the greatest readiness. Such a comparison was in progress at the time when a severe storm put a temporary end to the available supply of *Symphytum* flowers. Reserving, therefore, for a subsequent paper the details of the work already done and certain points requiring further elucidation, it may be said briefly that of all of the acids studied (carbonic, benzoic, salicylic, valeric, butyric, acetic, sulphuric and hydrochloric), carbonic is by far the most effective when pure solutions *of equal pH* are compared, in causing a visible change in intracellular acidity. For example, with a solution saturated with CO_2 at ordinary temperatures, a visible change in color usually begins in one or two minutes, while with the acids next in the order of their effectiveness (benzoic and valeric) fifteen to thirty minutes are required, with butyric, acetic and salicylic acids following in the order named. The mineral acids are only very slightly effective.

These results, as far as the acids other than carbonic (which apparently has not yet been studied in this manner) are concerned, agree fairly well with the findings of Haas (8) and of Collett (9) and also with the observation of the author (1) that tadpoles are fatally injured in saturated CO_2 solutions in two or three minutes, while in solutions of other acids of the same pH, from one to several hours are required to produce the same result.

If carbonic acid be compared with other acids of the same normality instead of the same pH, it appears far down the list, as would be expected, not because it does not enter cells readily—for there is much evidence that it does—but because, on account of its weakness (i.e., its low degree of dissociation), it is necessary for so much more of it to enter the cell before a change in the color of the indicator can occur than in the case of the more strongly dissociated acids.

One further point may be noted. If a solution, e.g., N/10, of NaOH have various acids added to it (in as concentrated a form as possible to avoid dilution) until the point of neutrality, as shown by phenolsulphonephthalein is reached, there should at this point, in the case of fairly strong acids, be practically no free acid present, while in the case of a weak acid like H_2CO_3, there should be a considerable amount. This difference may easily be made visible in the case of carbonic and other lipoid-soluble acids, such as benzoic, salicylic and butyric, by using the "artificial cells" already described. In one such experiment where the solutions were all neutral and N/10 with respect to total base, the indicator solution within the vial began to change color in three minutes with carbonic acid, while with butyric, benzoic and salicylic acids (all of which are highly effective with the "artificial cells" in the free form on account of their solubility in xylene) no change had occurred in three hours. Had the water used been so free from CO_2 as to make possible the use of an indicator solution with a lower buffer value, the effect of the smaller amounts of the other acids could probably have been detected as well, but as the experiment was not intended to be accurately quantitative, but merely to show that a very considerable difference exists between carbonic and the other acids, it was not considered necessary to take this precaution.

As the final conclusion to be drawn from the various experiments described in this paper, which have been made partly on living plant cells and partly on a simple "artificial cell," it may be stated that the physiological behavior of CO_2 probably depends to a considerable extent on two of its chemical and physical peculiarities: *a*, the weakness of H_2CO_3 as an acid, permitting the existence of a relatively large amount of the free but undissociated acid (as well as dissolved CO_2) in the equilibrium that exists at neutrality or slight alkalinity; and *b*, the readiness with which the undissociated acid, or its anhydride, CO_2, enters living cells, perhaps in virtue of its lipoid solubility. The combination of these two peculiarities is responsible for certain of the remarkable, and in some respects, unique, physiological properties of carbon dioxide.

SUMMARY

1. It has been found that the flowers of *Symphytum peregrinum* contain a natural indicator sensitive to carbonic acid, which may be used to study cell penetration by CO_2.

2. Using this material, it appears that a condition of intracellular acidity can be produced by a slightly alkaline solution of CO_2 in M/2 $NaHCO_3$ almost as effectively as by a solution of CO_2 in distilled water, though the hydrogen ion concentration of the latter solution is approximately four thousand times as great as that of the former.

3. A similar result may be obtained by the use of an "artificial cell" whose construction is described.

4. From pure aqueous solutions of acids of the same pH, carbonic acid changes the color of *Symphytum* flowers more quickly than do any of the other acids studied.

5. The ability of neutral and slightly alkaline solutions containing CO_2 to produce intracellular acidity is probably due to at least two factors: *a*, the weakness of H_2CO_3 as an acid and *b*, the lipoid solubility of CO_2 or H_2CO_3 or both, and the lack of such solubility in the case of bicarbonates.

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THE EFFECT OF SALT INGESTION ON CEREBRO-SPINAL FLUID PRESSURE AND BRAIN VOLUME

FREDERIC E. B. FOLEY AND TRACY JACKSON PUTNAM

From the Laboratory for Surgical Research, Harvard Medical School

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Recently Weed and McKibben (1), (2) demonstrated the important physiological fact that it is possible to reduce the cerebro-spinal fluid pressure and diminish the bulk of the brain by the simple procedure of injecting a hypertonic solution into the blood stream. Dr. Harvey Cushing, appreciating the clinical possibilities of this discovery, was led to suggest the present study on the following speculations:

> It is common knowledge that people who suffer from headaches suggesting "tension headaches" may get relief by a thorough intestinal evacuation, particularly when this is accomplished by salines. This has led to the view that constipation in itself is provocative of such discomforts. It would be of profit to repeat the observations of Weed and McKibben and then attempt to accomplish the same results by giving the hypertonic saline by mouth instead of intravenously. It seems possible that the withdrawal of fluid from the blood may increase its salt content sufficiently to cause the same result but without actually introducing salt into the blood stream. The fact established by Weed and McKibben might be adaptable to certain cranial operations, making them easier by lowering tension and diminishing brain volume.

INTRODUCTORY

In this laboratory some years ago, L. H. Weed began a series of experiments in an effort to answer certain questions relating to the cerebro-spinal fluid circulation which had been suggested by Doctor Cushing (3). His studies related largely to the circulation of the fluid and to the methods of its escape from the cerebral chamber (4). It was demonstrated that the normal escapes for fluid were by way of the arachnoid villi directly into the large dural sinuses and along the perineural spaces about the cranial and spinal nerves. He subsequently showed how the arachnoid spaces and dural sinuses developed (5).

On the side of pure physiology we are not so secure. It has recently been claimed that a solution of the primary problem of fluid production has not been accomplished. There is much presumptive evidence of an experimental and clinical nature that the choroid plexus behaves as a secreting gland in this connection. Such has been the generally accepted belief. Later students of the subject, and in particular Becht (6) and Becht and Matill (7), have objected to some of these generally accepted broad facts. They have recently published the results of a very critical reinvestigation of the subject and conclude that there is no indisputable evidence that the fluid is a secretory product of the choroid. Dixon and Halliburton (8), 1913, published an elaborate investigation of the action of certain drugs and tissue extracts on the "secretion." Their results are somewhat discounted by the work of Becht and Matill who point out that the results obtained may have been due to intracranial vascular (pressure and volume) changes caused by the injected substances and recorded as fluid secretion changes through the inadequacy of the methods employed. These authors applied the same criticism to that part of the work of Cushing and Weed (9) in which they claimed to demonstrate an increased secretion of fluid following the administration of hypophysis extract. The criticism does not seem warranted since in some of their experiments Cushing and Weed measured the increased flow of fluid from a catheter within the ventricle. The catheter was sealed off from the subarachnoid space by its contact with the walls of the aqueduct. Under such circumstances the changes in capacity of the subarachnoid space, incident to vascular changes, could not impart a significant alteration to the flow from the ventricular catheter.

Aside from the primary broad facts of anatomy, secretion and absorption channels, the work of Weed and McKibben brings out the most definite and significant fact established in regard to the detailed physiology and pharmacology of the cerebro-spinal fluid. The demonstration that intravenous injections of hypertonic solutions reduce cerebro-spinal fluid pressure and brain bulk offers a new point of departure into the problems of the physiology and pathology of the cerebro-spinal fluid.

A brief summary of the work of Weed and McKibben (loc. cit.) is essential to a presentation of the present study. In experiments on cats it was shown that hypertonic solutions of electrolytes injected into the venous circulation caused a marked fall of the cerebro-spinal fluid pressure. Hypertonic solutions of other crystalloids (glucose) caused a similar fall of pressure though not so great in extent. Saturated solutions of sodium chloride, sodium bicarbonate and sodium sulphate were employed. Conversely, it was found that distilled water (a hypotonic solution) had the opposite effect and caused a rise of pressure. It was later observed that the hypertonic solutions caused the brain to diminish in bulk while the hypotonic solution had the opposite effect, the brain increased in bulk. It was suggested that these changes were mediated through changes in the osmotic value of the blood.

EXPERIMENTAL

In experiments of this sort the methods employed are important to a proper interpretation of results. For this reason the method employed is set forth in considerable detail.

Animals. Most of the experiments were made on cats. The animals were taken from stock, usually without any preparation. In a few instances castor oil was given twenty-four hours before experiment. Some of the animals were allowed no food or water for this period. These measures did not appear to affect markedly the results and were given up. The animals were securely fixed in the prone position, the neck flexed and the head held tightly in place. For the recording of accurate pressure changes the fixation and position of the animal are most important for slight movements greatly affect the pressure.

Anesthetics. We have used the following anesthetics: Ether *a*, by cone and *b*, by the intratracheal method; *c*, morphine-atropine-urethane; *d*, luminal and *e*, morphine-chloretone. In our experience morphine-atropine-urethane is the most satisfactory anesthetic for the experiments on cats. The anesthesia requires no attention, it is uniform throughout the experiment and appears to have little effect on the fluid pressure. Chloretone does almost as well. With ether anesthesia the animals lose heat so rapidly that even with them placed on electric pads and under the warmth of several electric bulbs we have found it impossible to keep them in good condition for long periods of observation (8 to 10 hours). Changes in the depth of ether anesthesia markedly affect the intracranial fluid-blood pressure relationship causing changes quite apart from the purpose of experiment. The animals eventually fail when morphine-atropine-urethane is used, but in our experience it gives the most satisfactory period for observation.

Manometer and connections. The fluid pressures were read from a U-tube manometer connected to a needle in the subarchnoid space. The method of establishing the manometer connection is of importance. The whole system was filled with normal saline, the column of fluid being set at 100 mm., somewhat below the initial pressure usually to be anticipated. A wire obturator extended through a water-tight puncture in the tubing on into the needle. With the apparatus so arranged the puncture through the occipito-atlantoid ligament was made. The obturator was withdrawn from the needle but its end was still left in the tubing to occlude the puncture through which it was inserted. By this method we were able to avoid the great loss of fluid and artificial fall of pressure incident to beginning with an empty system into which the fluid must rise to fill the manometer. The bore of the manometer was such that 100 mm. pressure change required a displacement of 0.26 cc. of fluid.

The importance of the displaced fluid in experiments of this sort has been emphasized. It will vary directly with the bore of the manometer. The possible bearing of this fact on the results was realized and so a number of control experiments with a larger bore manometer were conducted in which 100 mm. pressure change required a displacement of 1.06 cc. of fluid.

Arterial and venous pressures. These were recorded in some of the first experiments. It soon became apparent that they were not essential to an interpretation of the enormous fluid pressure changes that occurred and so their recording was given up.

Routes of administration: Intravenous doses were given from a buret through a cannula in the femoral vein. It was possible to control accurately the rate of flow. *Gastro-intestinal doses* were given through a stomach tube. *Duodenal doses* were given by a syringe and needle into a loop of duodenum exposed through a small mid-line incision. *Colonic doses* were given by a rectal tube held in place by a purse string suture in the anus.

Normal pressures. We wish first to record the values we have obtained for normal pressures. In addition to the present series Weed and McKibben (loc. cit.) and Becht (loc. cit.) furnish the most comprehensive data on the subject. In a series of one hundred experiments on cats under the experimental conditions described, the average of normal pressures of the cerebro-spinal fluid was 133 mm. normal saline. This is 14 mm. higher than the value of 119 mm. given by Weed and McKibben and 21 mm. higher than the value of 112 mm. given by Becht. The former authors cited used an empty manometer into which the fluid had to rise. It is not clear whether or not Becht began with a filled system in the experiments on which this figure is based. At any rate, this factor in our experiments probably, in part, explains the higher value of our normal pressures. When an appreciable amount of fluid has been displaced it takes a considerable period of time for a new equilibrium to be established. Under these circumstances readings taken early in an experiment only represent a point in the transition toward equilibrium. The average normal with ether anesthesia was 127; with urethane 155 and with chloretone 127. The higher value of our normal readings may be connected with the use of urethane. The highest initial pressure we recorded was 255 mm. The lowest positive pressure was 65 mm. In three animals the pressure at the beginning of experiment was negative, viz., −10 mm., −20 mm., −25 mm. These values existed after the 100 mm. column of fluid in the manometer (0.26 cc.) had run into the subarachnoid space. The experimental details were carefully checked up in these cases so that we are sure the figures represent true values.

INTRAVENOUS DOSES

A number of experiments in which the solutions were given intravenously were carried out. They abundantly confirmed the work of Weed and McKibben (loc. cit.). In addition they furnished a basis for comparison between intravenous and gastro-intestinal doses of the solutions.

Ringer's solution regularly caused a marked though unsustained rise of the cerebro-spinal fluid pressure. For example an intravenous dose of 50 cc. was followed by a rise of the cerebro-spinal fluid pressure of 100 mm. Within an hour the pressure had fallen to approximately a normal value again. On the other hand, the same amount of water given intravenously caused a marked and sustained rise of the cerebrospinal fluid pressure (100 mm.) which, an hour after the injection, was still at its height. These changes were not accompanied by significant changes in the blood pressure. Figure 1 illustrates the effect on cerebro-spinal fluid pressure and blood pressure of an intravenous injection of 25 cc. 30 per cent sodium chloride solution. The cerebro-spinal fluid pressure promptly fell from 175 mm. to −78 mm. The fall is sustained, for two hours and fifteen minutes after injection the pressure remains 197 mm. below its level at the beginning of experiment.

These experiments are typical of our results with intravenous injections. The changes are greater than those recorded by Weed and McKibben but qualitatively they are identical with their experiments. In longer continued experiments we have found the lowered cerebro-spinal fluid pressure persisting for long periods. In one experiment, for example (10 cc. 30 per cent NaCl intravenously), it remained 110 mm. below its initial level five hours and twenty minutes after injection. In this experiment a fall of 238 mm. took place in fifty-four minutes or at the rate of approximately 4.4 mm. per minute. The return toward normal occurred more slowly, at the rate of approximately 0.7 mm. per minute.

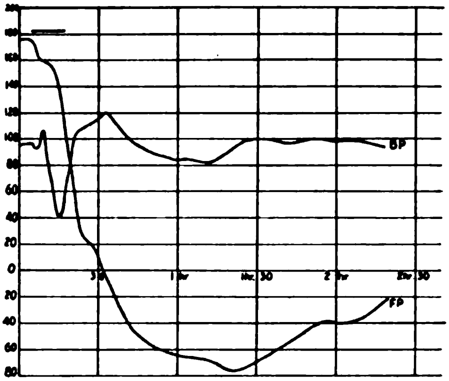

Fig. 1. Intravenous injection of 25 cc. 30 per cent sodium chloride solution. Dog weight 6.0 kgm. Ether by cone. Cerebro-spinal fluid pressure (mm. saline) and blood pressure (mm. Hg.) curves. During the injection there was marked cardiac and respiratory disturbance—the respirations became rapid and shallow, almost ceasing, the heart rate rose and the beats became feeble. Note the 70 mm. fall of arterial pressure during this period. A prompt recovery occurred with return to a higher value than previously. An abrupt fall, 253 mm. of cerebro-spinal fluid pressure occurred. Two hours and fifteen minutes after injection this pressure remains at −22 mm. or 197 mm. below the level at the beginning of experiment. The change is independent of blood pressure.

Unless the rate of injection of the hypertonic sodium chloride is carefully controlled the cardiac and respiratory changes may prove fatal. Death from these causes occurred in several experiments.

GASTRO-INTESTINAL DOSES

General effect. By a single experiment our main anticipation in regard to salt ingestion was shown to be well warranted. To an anesthetized dog a massive dose (180 cc.) of 30 per cent sodium chloride was given by stomach tube. The cerebro-spinal fluid pressure promptly fell from 150 mm. (normal saline) to zero. This was accompanied by a rising arterial pressure during the whole experiment. This is the essential fact. The subsidiary facts—size of dose, response to different substances, vascular changes, duration of the effect and so on—will be presented in the following experiments.

Size of dose. Figure 2 illustrates the effect on cerebro-spinal fluid pressure and blood pressure of injecting 35 cc. 10 per cent sodium chloride solution into the rectum. Five minutes after the injection was begun the cerebro-spinal fluid pressure began to fall. In one hour and ten minutes it had fallen from 112 mm. to −44 mm., a drop of 156 mm. This was at the rate of 2.2 mm. per minute. The change is quite independent of the blood pressure which, as may be seen, remains quite constant. The change here is not so extensive nor rapid as in the case of intravenous doses—compare figure 1 with figure 2.

That the extent and rate of change obtained in this experiment are not the maximal ones to be obtained by intestinal doses was soon apparent. Figure 3 illustrates this fact. In this experiment 50 cc. of 30 per cent sodium chloride solution were put into the rectum. The fall of pressure and the rate at which it occurred were here equal to intravenous doses, a fall of 270 mm. at the rate of 4.2 mm. per minute, compare figure 1 with figure 3.

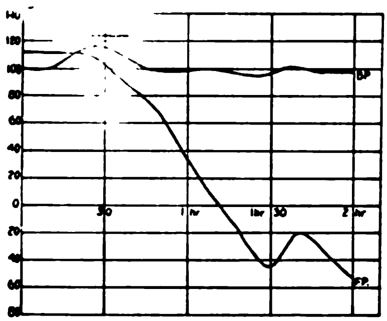

Fig. 2. Thirty-five cubic centimeters 10 per cent sodium chloride solution by rectal tube. Cat weight 2.5 kgm. Intratracheal ether. Cerebro-spinal fluid pressure (mm. saline) and blood pressure (mm. Hg.). Soon after the injection is begun the cerebro-spinal fluid pressure begins to fall—156 mm. in 1 hour and 10 minutes. Rate 2.2 mm. per minute. The arterial pressure rises a little during the injection but shows no significant change.

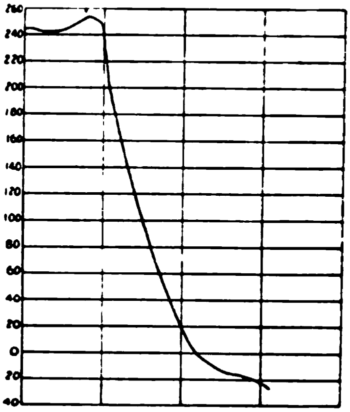

Fig. 3. Fifty cubic centimeters 30 per cent sodium chloride by rectal tube. Cat weight 3.2 kgm. Intratracheal ether. A fall of 270 mm. occurred at the rate of 4.2 mm. per minute. The curve closely resembles that obtained with intravenous doses, an example of which is shown in figure 1.

Changes of this extent are maximal and larger doses add nothing to the effect. Much smaller doses, 20, 10, 5 cc. of 30 per cent salt solution still cause very marked falls of pressure. An injection of 5 cc. of 30 per cent sodium chloride solution into the duodenum caused a fall of 104 mm. in the cerebro-spinal fluid pressure. Comparing this with the injection of a similar amount of the same solution intravenously, little difference between the effects produced is found. The intravenous dose causes a slightly more rapid fall.

Route of administration. As between rectal, duodenal and gastric doses little difference in the effect is to be noted when large doses are employed. Smaller doses, however, are most efficient in the duodenum.

Concentration of solution. Solutions of much lower concentrations than those mentioned so far are capable of producing changes of the same sort. Here, however, the rate of change is much slower and the fall of pressure is decidedly less in extent. In a number of experiments the average maximal effect to be obtained with large doses (40, 50, 150 cc.) of 2 per cent sodium chloride per duodenum was a fall of 97 mm. pressure at the average rate of 2.5 mm. per minute. Figure 4 represents an experiment of this sort. The average maximal effect obtained with the large doses (20 to 35 cc.) of 30 per cent sodium chloride on the other hand was a fall of 258 mm. pressure at the average rate of 3 mm. per minute.

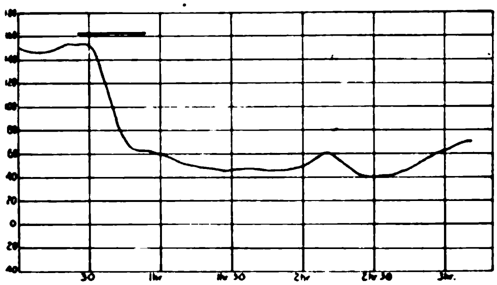

Fig. 4. Injection of 150 cc. 2 per cent sodium chloride solution, containing 2.7 per cent magnesium sulphate into the duodenum. Cat weight 3 kgm. Urethane anesthesia. Curve of the cerebro-spinal fluid pressure changes (mm. saline).

The large volume of solution in the case of the doses of lower concentration probably brought the solution into contact with a larger area of intestinal mucosa than was the case with the smaller volume of solution when 30 per cent salt was used. Were it not for this factor, the lower concentrations would have very likely affected the change at a still slower rate.

Comparison with intravenous doses. These large doses of concentrated salt solution in the gastro-intestinal tract cause maximal effects as regards the extent of the fall of pressure. Comparing the results of such experiments with those in which the intravenous route was used the extent of fall in the two cases is approximately equal. The intravenous doses produce the effect at a slightly more rapid rate.

Duration of effect. Some of the experiments were continued for long periods. It was found that the lowered pressures endured for a considerable period. The curves are too long for reproduction but the following tabulation of four experiments (table 1) illustrates the point.

TABLE 1

Cerebro-spinal fluid pressures at hour intervals following salt ingestion

	INITIAL	HOUR INTERVALS								
		1	2	3	4	5	6	7	8	9
10 cc. 30 per cent NaCl.	160	−78	−35	+4	+32	+45				
20 cc. 30 per cent NaCl.	240	−40	−50	0	+60	+110	+140	+130	+150	+145
20 cc. 30 per cent NaCl.	120	−70	−85	−40	−2	+20	+38	+55		
10 cc. 30 per cent NaCl.	230	0	+40	+84	+138	+182				

TABLE 2

Cerebro-spinal fluid pressures at long intervals after salt ingestion

	PRESSURE WHEN PUNCTURED	NORMAL	DIFFERENCE
20 cc. 30 per cent NaCl 48 hours before puncture 20 cc. 30 per cent NaCl 24 hours before puncture....	100	133	33
15 cc. 30 per cent NaCl 22 hours before puncture 15 cc. 30 per cent NaCl 18 hours before puncture....	75	133	58
35 cc. 30 per cent NaCl 17 hours before puncture.....	55	133	78
35 cc. 30 per cent NaCl 22 hours before puncture.....	123	133	10
Average..	88+	133	45−

We felt that possibly under the experimental conditions, anesthesia etc., it was impossible for the pressures to recover. For this reason other animals were given doses of 30 per cent sodium chloride or saturated Na_2SO_4 the day previous to experiment. The following day their pressures were determined. As we did not know their pressure before the ingestion of salt, the results are not of great value. The pressures found the next day can only be compared with our average normal pressures for animals under similar experimental conditions. The data bearing on this subject are given in table 2.

In these four experiments the average of pressures found many hours after salt ingestion is 88 mm., i.e., 45 mm. lower than our average of 133 mm. in the normal animal.

Unabsorbable salts. Experiments in which the unabsorbable salt, sodium sulphate, was used were also carried out. Changes qualitatively like those following sodium chloride occurred. The extent of change, however, and the rate at which it occurred were somewhat smaller in the case of the sulphate. Figure 5 is plotted from a sulphate ingestion experiment, a fall of 202 mm. occurred at the rate of 2.2 mm. per minute. Compare with figure 3, an experiment in which a large dose of sodium chloride was given. The average extent of fall after large doses of sulphate was 122 mm. at the average rate of 2.4 mm. per minute. Smaller doses caused roughly proportionate falls of pressure. As in the case of chloride ingestion, the low pressures endure for a considerable period.

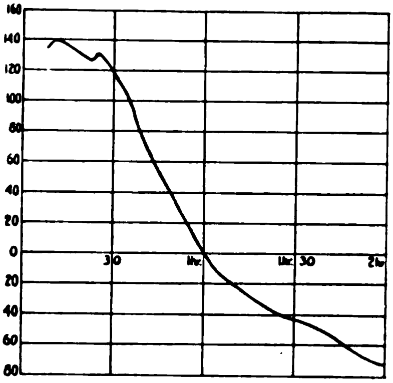

Fig. 5. Injection of 35 cc. saturated (120° F.) sodium sulphate solution into the duodenum. Cat weight 3.5 kgm. Chloretone anesthesia. A prompt fall of cerebro-spinal fluid pressure occurred—202 mm. at the rate of 2.2 mm. per minute. This rate is somewhat slower than in the case of similar doses of sodium chloride.

This ingestion of salt does not appear to make the animals refractory to a second dose at a later time. This was demonstrated by administering the salt on two successive days. Following the second administration the typical pressure changes occurred.

Glucose solutions. Concentrated solutions of glucose given into the gastro-intestinal tract are capable of producing qualitatively these same results, though quantitatively smaller in extent. Thus following the administration of 35 cc. of concentrated glucose solution into the duodenum the cerebro-spinal fluid pressure fell 140 mm. This is the largest fall we have obtained with dextrose solutions. The results are not as constant as with the salts.

Hypotonic solutions. The effects produced with hypotonic solutions are not nearly so striking. Several experiments were conducted in which water was injected into the duodenum. Thirty-five cubic centimeters caused rises of pressure averaging 45 mm. This rise is not very well sustained for within an hour practically normal pressure values obtained. Water seems somewhat more effective when it follows by several hours the ingestion of a hypertonic solution.

Alteration of brain bulk. A number of observations were made on the change of brain bulk following the administration of 30 per cent sodium chloride solution. The method employed was like that described by Weed and McKibben. The temporal muscles were dissected back and the dura with the underlying brain was exposed, through two trephine openings. The sodium chloride solution was injected into the duodenum. Within a short time following the administration of the solution the brain began to recede from the margins of the skull. Previously it had been bulging under the tense dura. The changes were very obvious, for within twenty to forty minutes the bulging had completely disappeared and the surface was concave. On incising the dura at this stage the sulci were found to be quite broad while the convolutions were contracted and narrow.

Proof of secretion and absorption pressure changes. The question naturally arises as to whether or not, in experiments like those recorded, the changes in the height of the manometer column may not be due simply to the dislocation of fluid from the manometer into the cerebrospinal fluid spaces coincident with the diminution in the size of the brain. That this factor plays some part in the pressure changes recorded, there can be no doubt for obviously as the subarachnoid spaces enlarge with a diminishing brain volume, fluid must run in to occupy them. On the other hand, there is very good evidence that the ingestion of these solutions causes alterations in the ratio between secretion and absorption of the fluid, producing pressure changes quite independent of brain volume changes. This fact was demonstrated by a number of experiments. With a certain dose of hypertonic solution the pressure changes of the cerebro-spinal fluid were recorded in a manometer of such bore that 100 mm. pressure change required a displacement of 0.26 cc. of fluid. The pressure changes following this same dose were then recorded in another animal using a larger bore manometer, in which 100 mm. of pressure change required a displacement of 1.06 cc. of fluid. Figure 6 shows the two curves obtained. If the whole process were merely one of change in brain volume, the second curve (large bore manometer, broken line) should illustrate a fall of pressure at a very much slower rate and very much less in extent, for the fluid displaced into the subarachnoid spaces would change the height of the column in the small bore manometer a great deal more than in the large bore manometer. Such is not the case, for the new ratio established between secretion and absorption is capable of maintaining the pressure at a new level, independently of the volume of the cerebro-spinal fluid spaces or the size of the manometer used. A later report will be made of the remarkable changes which these solutions bring about in the absorption mechanism and in the currents of the fluid channels. They appear to be capable of reversing the flow in the perivascular spaces and the ventricular system.

The clinical significance of these problems is most apparent. They concern the states commonly referred to as "pressure symptoms." Up to the present the facts established by research have been more anatomical than physiological and it is significant that the clinical advances have been mostly applications of these facts and have little to do with physiology.

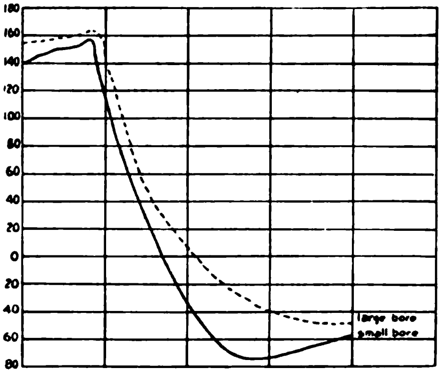

Fig. 6. Curves from two separate experiments. The broken line describes the cerebro-spinal fluid pressure as recorded in a large bore manometer, the continuous line describes the same pressure changes in another animal, but recorded with a small bore manometer. In each case the animal (cat) was given 10 cc. of 30 per cent sodium chloride solution intravenously.

SUMMARY AND CONCLUSIONS

1. Weed and McKibben demonstrated that it is possible to reduce the cerebro-spinal fluid pressure and diminish the bulk of the brain by injecting hypertonic solutions into the blood stream. Conversely, they showed that the pressure and the bulk of the brain could be increased by the injection of hypotonic solutions. Their work has been repeated and their general conclusions confirmed.

2. We have shown that the introduction of hypertonic salt solutions into the gastro-intestinal tract has a similar effect. This route of administration is more convenient, and by its use the disturbances of circulation and respiration common with intravenous infusions are avoided.

3. Twenty to thirty cubic centimeters of a 30 per cent sodium chloride solution introduced into the duodenum or rectum of an average

sized cat produced a maximal fall of cerebro-spinal fluid pressure. Following such doses, the average fall of pressure in a large series of experiments was 258 mm. of water. Larger doses added nothing to the extent of the fall. A dose of 5 cc. produced a fall of 104 mm. of water, and intermediate doses gave roughly proportionate results. Following this fall in pressure there is a gradual rise. Thus, seventeen to forty-eight hours after such injections, four animals showed pressures averaging 45 mm. less than the average normal.

4. Sodium chloride solutions in only slightly hypertonic concentration are also effective in causing a fall in cerebro-spinal fluid pressure, although to produce appreciable changes, large doses are required. Doses of 40 to 150 cc. of 2 per cent sodium chloride solution caused falls of pressure averaging 97 mm. of water, in cats.

5. Saturated solutions of sodium sulphate, which is not absorbed from the gastro-intestinal tract, produced qualitatively similar results, but less in extent and at a slower rate. With concentrated dextrose solutions the fall is still less and its rate still slower.

6. Water injected into the duodenum produces a small rise of pressure in the normal animal, but it disappears more rapidly than the fall incident to salt ingestion. If the animal has been given a concentrated saline solution the day before, the rise in pressure following the administration of water is more marked and of longer duration.

7. Such changes in cerebro-spinal fluid pressure were shown to be independent of changes in arterial or venous blood pressure.

8. These changes of fluid pressure are accompanied by a decrease in the size of the brain.

9. The manometer readings (pressure values) obtained after salt ingestion are not due solely to changes in brain volume and capacity of the cerebro-spinal fluid spaces, but primarily represent new ratios between secretion and absorption of cerebro-spinal fluid.

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ANTAGONISM OF DEPRESSOR ACTION OF SMALL DOSES OF ADRENALIN BY TISSUE EXTRACTS

J. B. COLLIP

From the Department of Biochemistry and Physiology, the University of Alberta, Edmonton, Alberta, Canada

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In a previous communication the writer (1) has shown that extracts made from heart, spleen, pancreas, testes, anterior and posterior lobe of the pituitary body, and the thymus, thyroid and parathyroid glands, in addition to stimulating the isolated uterus of the rat, guinea pig, virgin dog and cat, antagonized the inhibitory action of adrenalin on this tissue. It was also shown that the same extracts depress the cardiac vagus of the terrapin. It was demonstrated also that the pressor effect following the intravenous injection of a definite dose of adrenalin was augmented and longer maintained when splenic extract was previously injected. It was suggested that this latter result might be due to the depression or paralysis of some part of the vasodilator mechanism by the splenic extract.

Further investigation has confirmed the writer in this opinion.

Methods. The extracts used were made by boiling with distilled water in amount sufficient to give a practically isotonic solution, desiccated glandular products supplied by Armour & Company, Chicago; or else by extracting fresh tissue with distilled water on the water-bath, filtering and concentrating the filtrate to an aliquot volume of the original tissue.

The animals used for testing out the extracts were the dog and rabbit. The experimental animal was placed under ether anesthesia and a cannula was inserted into the left carotid artery. This was connected with the mercury manometer. Injections were made into the left external jugular vein by means of a "Record" syringe. If the fluid injected were small in amount the vein was at once flushed with Ringer-Locke's solution to insure that all the fluid should enter the general circulation.

Results. It was found that extracts of such widely divergent tissues as those of the spleen, skeletal muscle and parathyroid gland caused the pressor response of a definite dose of adrenalin administered by the intravenous route to be augmented and longer maintained. Thus it was found that 3 cc. of adrenalin, 1:50,000, injected into a dog weighing 14 kilos, produced a rise of 32 mm. in blood pressure with a return to normal in 40 seconds, whereas the same amount of adrenalin injected 5 minutes after the administration of 25 cc. of extract of ox spleen produced a rise of 52 mm. in blood pressure with a return to normal in 4 minutes. The injection of 0.5 cc. of 1:50,000 adrenalin into a rabbit weighing 2 kilos produced a rise of 54 mm. in blood pressure with a return to normal in 80 seconds while the same amount given after the injection of 5 cc. of extract of ox muscle produced a rise of 62 mm. in blood pressure with a return to normal in 3½ minutes.

In another instance a dog weighing 22 kilos with both vagi cut responded to 4 cc. of adrenalin 1:50,000 by a rise of 80 mm. in blood pressure with a return to normal in 1 minute 50 seconds; 20 cc. of extract of ox spleen were then injected and when the depressor action of this had passed off 4 cc. of adrenalin 1:50,000 were again injected. This produced a rise of 110 mm. in the blood pressure with a return to normal in 3 minutes.

An animal in which the depressor effect of small doses of adrenalin is readily elicited is most suitable to demonstrate the antagonism of this latter action by tissue extract. Figure 1 illustrates the antagonism of the vasodilator action of small doses of adrenalin by extracts of various tissues. The experimental animal in this instance was a male dog 25 kilos in weight. Shortly after ether anesthesia had been induced, the injection of 2 cc. of 1:50,000 adrenalin produced a fall of 22 mm. of mercury in the blood pressure (fig. 1, I). Both vagi were cut and the injection of 3 cc. of 1:50,000 adrenalin produced a fall of 32 mm. in the blood pressure. One cubic centimeter of 1:1,000 adrenalin was next injected and the blood pressure rose 86 mm. The injection of 3 cc. of 1:50,000 adrenalin then produced a slight rise in the blood pressure which was followed at once by a slight fall in the same with an immediate return to normal. This latter effect stands in sharp contrast with the effect of 3 cc. of 1:50,000 adrenalin prior to the injection of the stronger extract (fig. 1, A). After 10 minutes had elapsed the injection of 3 cc. of 1:50,000 adrenalin produced an effect similar to that following the first injection of 3 cc. Figure 1, B, shows the conversion of a fall of 16 mm. in blood pressure following the intravenous injection of 3 cc.

of 1:50,000 adrenalin into a rise of 20 mm. after the injection of 10 cc. of extract of thyroid gland.

Figure 1 (*C*, *D*, *E*, *F*, *G* and *H*) illustrates the same type of phenomenon induced by extracts of pancreas, thymus gland, corpora lutea, anterior lobe of pituitary, testes and parathyroid glands respectively.

This antagonism of the depressor action of small doses of adrenalin by extracts of various tissues was not of long duration. The primary effect of adrenalin in small doses given intravenously returned in from 10 to 30 minutes after the injection of the tissue extract (fig. 1, D and G).

Discussion. The antagonism of the depressor action of small doses of adrenalin and the augmentation and prolongation of the pressor response to the larger doses would appear to be phenomena of the same order. As Hartman (2) and others have shown, there is a definite vasodilator mechanism which is activated by adrenalin. Different opinions have been expressed as to the exact causation of adrenalin dilatation. Dale (3) was of opinion that small amounts of adrenalin stimulate the vasodilator endings. Cannon and Lyman (4) attributed the two effects, vasodilatation and vasoconstriction, to opposite actions according to the state of the muscle,—relaxation when tonically shortened, contraction when relaxed. Gruber (5) suggested that the central nervous system was involved in the dilatation from adrenalin. Hartman, Kilborn and Fraser (6) obtained vasodilatation in the hind limb and intestine by the central action of adrenalin. They concluded that adrenalin stimulates vasodilator cells situated in the sympathetic and posterior spinal root ganglia.

It would appear that the tissue extracts studied depress in some manner some part of the vasodilator mechanism. The effect of a small dose of adrenalin which normally produces a fall in blood pressure is thus neutralized in whole or in part by the tissue extract as far as the dilator action is concerned, and constriction only or else a lesser degree of dilatation takes place. When a larger dose of adrenalin is given, one such as causes a rise in blood pressure, the administration of tissue extract depresses the vasodilator apparatus and the vasoconstrictor effect is thus augmented and prolonged. Whether the point of action of this peculiar constituent of tissue extract is the nerve ending, nerve fiber or nerve cell of the vasodilator apparatus is undetermined. The writer inclines to the view that the action is peripheral since the antagonism of the inhibitory action of adrenalin on the uterus and the depression of the cardiac vagus of the terrapin produced by similar extracts was shown to be due to peripheral action (1). It is however possible that the vasoconstrictor

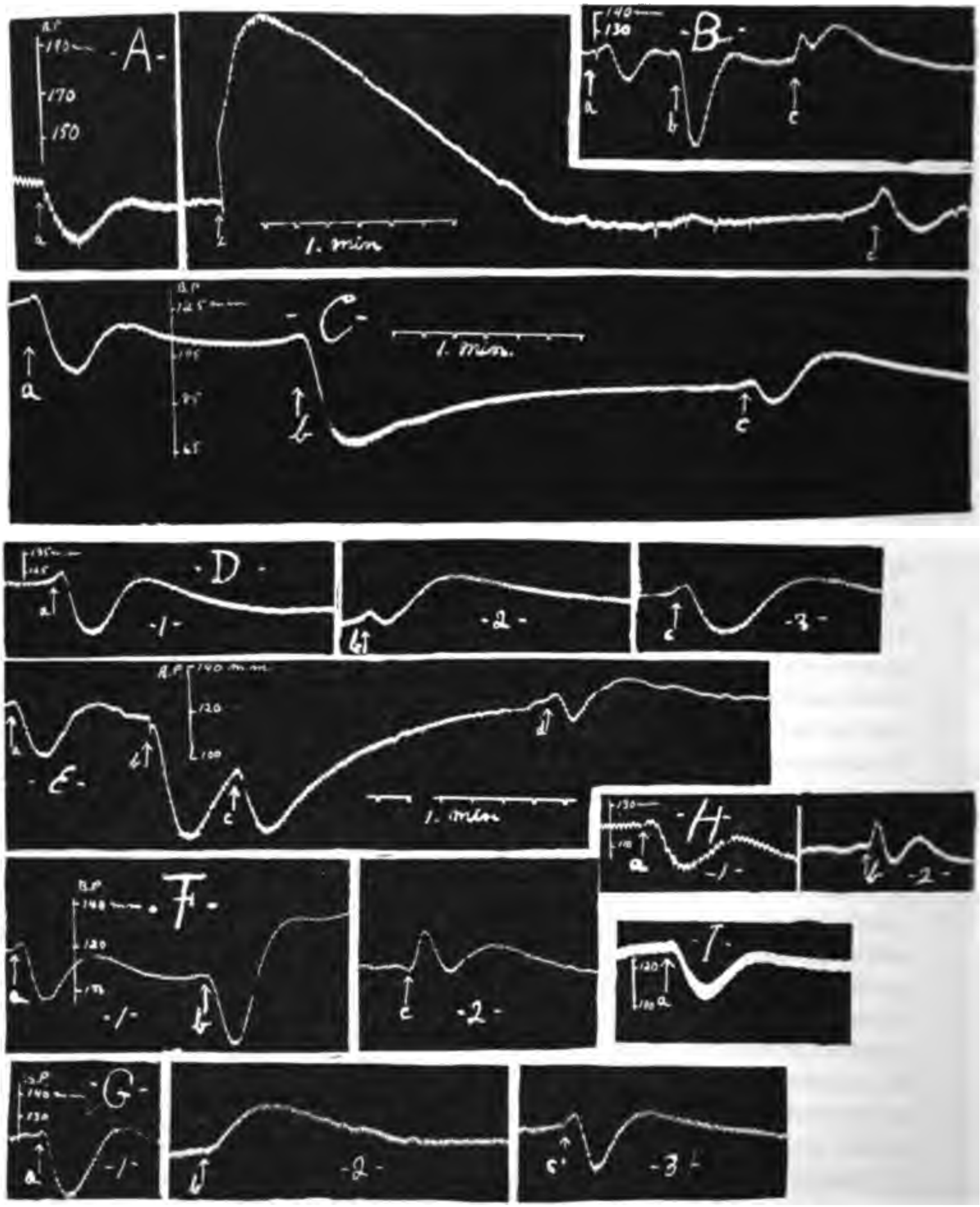

Fig. 1. Dog 0; 25 kilos. Ether anesthesia

A: *a*, 3 cc. of adrenalin 1:50,000 injected into left external jugular vein.
b, 1 cc. of adrenalin 1:1000 intravenous.
c, 3 cc. of adrenalin 1:50,000 intravenous.

mechanism may be rendered more sensitive to adrenalin by tissue extract.

The short duration of the antagonistic action of tissue extract, given by intravenous injection, upon adrenalin dilatation indicates that the active principle is either neutralized or destroyed fairly rapidly when present in the general circulation.

SUMMARY

1. The fall in blood pressure produced by a small dose of adrenalin is antagonized by various tissue extracts.
2. The rise in blood pressure produced by a definite dose of adrenalin is augmented and prolonged by administration of tissue extract.
3. It is held that both these types of effects are of the same order.

B: *a*, 3 cc. of adrenalin 1:50,000 intravenous.
b, 10 cc. extract of thyroid gland intravenous.
c, 3 cc. of adrenalin 1:50,000 intravenous.

C: *a*, 3 cc. of adrenalin 1:50,000 intravenous.
b, 20 cc. extract of pancreas intravenous.
c, 3 cc. adrenalin 1:50,000 intravenous.

D: *a*, *b* and *c*, 3 cc. of adrenalin 1:50,000 intravenous.
Between *1* and *2*, 20 cc. of extract of thymus gland injected into left external jugular vein.
3, five minutes after *2*.

E: *a*, 3 cc. adrenalin 1:50,000 intravenous.
b, 20 cc. of extract of corpora lutea intravenous.
c, 10 cc. of extract of corpora lutea intravenous.
d, 3 cc. of adrenalin 1:50,000 intravenous.

F: *a*, 3 cc. of adrenalin 1:50,000 intravenous.
b, 10 cc. of extract of anterior lobe pituitary intravenous.
c, 3 cc. of adrenalin 1:50,000 intravenous.
2, taken eight minutes after *1*.

G: *a*, *b* and *c*, 3 cc. of adrenalin 1:50,000 intravenous.
Between *1* and *2*, 40 cc. of extract of testes injected into left external jugular vein.
3, taken ten minutes after *2*.

H: *a* and *b*, 3 cc. of adrenalin 1 : 50,000 intravenous.
Between *1* and *2*, 10 cc. of extract of parathyroid glands injected into left external jugular vein.

I: *a*, 2 cc. of adrenalin 1:50,000 intravenous. Vagi intact.

Note: In all other instances vagi were cut.

4. The antagonism of the depressor action of adrenalin by tissue extract is of short duration.

5. Some part of the vasodilator mechanism is depressed by tissue extract.

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OBSERVATIONS ON A SEX DIFFERENCE IN THE PRESENCE OF NATURAL HEMOLYSIN IN THE RAT

YOSHIO SUZUKI

From the Wistar Institute of Anatomy and Biology

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It has been often found that without previous treatment the serum of one animal can hemolyse the red blood corpuscles from an animal of another species. Such hemolysin is called "natural hemolysin." The instances of the presence or absence of natural hemolysins against different species of animals have been noted among the various animals which are commonly used in the laboratories. However, so far as I am aware, no systematic observations have been made with the corpuscles or serum of the rat in regard to the hemolytic activities of these fractions against those from other animals, and for this reason we have carried out the following investigation.

The technique used is as follows:—The rat was lightly etherized and the blood was collected from the carotid artery. The serum was diluted to ten times its volume and in each case 0.2 cc. was mixed with 0.5 cc. of 10 per cent washed blood corpuscles of the pig. To this mixture 1.3 cc. of normal salt solution was added, thus making up the total amount to 2 cc. This mixture was placed for half an hour in an incubator at 37°C. Sometimes a much greater dilution of serum was also tried, but unless otherwise stated the results refer to the ten-times dilution.

It was found in the first instance that the rat serum did not hemolyse the red blood cells of the pig; in other words the rat serum did not possess the anti-pig natural hemolysin. This result was obtained from the mixed sera of a dozen adult rats of both sexes combined.

However during further experimentation it was accidentally noted in one case that the serum of the rat used when mixed with the pig corpuscles hemolysed the latter readily. This particular rat happened to be a female immediately after parturition. Since we had previously found, as we thought, that the rat serum lacks the anti-pig hemolysin, but yet in this particular instance a highly active anti-pig hemolysin was found, we decided at once to repeat the observations. Table 1 gives the results of the further observations.

We find from table 1 that during the first five to six days after parturition the anti-pig hemolysin is present, but is less frequently present after this period. This interesting result induced us to study further. As a first step we tested for the presence or absence of anti-pig natural hemolysin of rats under various conditions, and obtained the results which are shown in table 2.

TABLE 1

After parturition. Anti-pig natural hemolysins in female rat

DAYS AFTER PARTURITION	BODY WEIGHT	BODY LENGTH	AGE	HEMOLYSIN	REMARKS
	grams	*mm.*	*days*		
1				++	Normal
2 (2)				++++	Normal
2			150	+	Normal
3	171		90	+++	Normal
3			300	+	Normal
5	180		90	++++	Normal
5 (3)				–	Normal
6	168			++++	Slightly infected
9 (2)				–	Normal
10	115			–	Normal
11	171	184		++	Normal
16	131	179		–	Normal
25	165	183		–	Normal

TABLE 2

Natural anti-pig hemolysin in the rat serum under various conditions

Total number of rats examined = 211

PRESENCE OF ANTI-PIG NATURAL HEMOLYSIS IN RAT SERUM	FEMALES		MALES	
	Absent	Present	Present	Absent
	per cent	*per cent*	*per cent*	*per cent*
Pregnancy	35.8	64.2		
1 to 7 days after parturition	27.3	72.7		
7 to 25 days after parturition	83.3	16.7		
Normal adults	57.7	42.3	17.6	82.4
Normal young—under 50 days	87.5	12.5	14.3	85.7
Infected lungs	59.3	40.7	50.0	50.0

1. The number of cases in which the serum possesses the anti-pig hemolysin is greater among the females in pregnancy, during the first week after parturition, and in males in infection of the lungs.

2. The hemolysin is usually absent in rats of both sexes whose ages are less than fifty days.

3. Among the adult females the hemolysin is found in almost half the number of rats examined. Whether or not this high frequency was due to the use of rats at an early period of pregnancy cannot be determined. These females however had been with the males.

TABLE 3

Anti-pig natural hemolysin. Observations on the same animal at two or more ages

SEX	AGE AT THE TIME OF EXAMINATION				REMARKS
	30 days	70 days			
3 ♂	−	−			
♂	−	+			
♀	−	+++			
♀	−	++*			* Emaciated
♀	−	(+)			
♀	−	++			
	75 days	96 days			
♂	−	−			
♀	−	+*			* Pregnant
3 ♀	−	−			
	106 days	119 days	132 days	166 days	
♂	−	−	+ (sick)	+*	* Sick, killed
♀	++	++++			Sick, killed
♀	±	±			Died after operation
♀	−	−	−		
♀	−	+			Found dead
♀	+++	Not exam.	+++	+++	
	216 days	232 days			
♂	+	++			Sick, killed
♂	−	+			
♂	++++	+++			
2 ♀	−	−			
♀	−	+++			Sick, killed

4. Among normal males we find a comparatively small number of cases in which the hemolysin is present.

5. Among males with infected lungs, 50 per cent of the cases possessed the hemolysin.

We may conclude from these data that anti-pig natural hemolysin is far more frequently present in the female than in the male.

In order to verify the statistical findings, and also further to investigate whether or not the natural hemolysin appears suddenly associated with both pregnancy, as well as with lung infection, and also with the idea of determining whether or not the degree of titration increases with the advance of pregnancy or of infection, the following experiment was undertaken.

The rats at various ages were examined individually for the presence or absence of the anti-pig natural hemolysin. The same rats were again examined after a lapse of several weeks. In each instance the blood was obtained by puncture of the heart. As table 3 shows, this puncture was made in two instances at four different ages.

It was intended to carry such an examination throughout the entire span of life. However disturbances in the rat colony from ecto-parasites unfortunately prevented the completion of this work. Nevertheless the results obtained, though imperfect, show several interesting points, and these are presented in table 3.

From this table 3 we see that in many instances the hemolysin was not present at the earlier age but appeared later. In every instance where the hemolysin was found at the earlier age it was found later also.

DISCUSSION

Recently Kolmer and Casselman (1) examined a large number of human sera for natural hemolysins against the erythrocytes of the sheep, dog, ox, goat, hog, rat, chicken, horse, rabbit and guinea pig. They found a considerable variation in the activity of the human sera to these various blood cells. Kolmer and Casselman did not however mention any variation associated with either sex, disease or pregnancy.

While the present investigation was nearing completion, I received from Doctor Obata a paper (in Japanese) on comparative immunological studies on the fetal and adult sera in man. Doctor Obata found in man practically all that I have found in the rat; that is the anti-sheep natural hemolysin occurs more frequently in women than in men, and furthermore the hemolysin is not present in the fetal blood. Doctor Obata found however that the anti-sheep natural hemolysin is equally distributed among non-pregnant women and pregnant women, while my own observation on the rat shows a much greater frequency in both pregnancy and the first week after parturition than in the non-pregnant adult female.

From the foregoing observations I conclude that

1. Anti-pig natural hemolysin is not usually present in the serum of younger albino rats under thirty to fifty days of age.
2. In older rats this hemolysin may be present in the serum of both sexes but more frequently in that of the female than in that of the male rat.
3. During pregnancy, as well as during the first week after parturition, the anti-pig hemolysin is not only more active but the proportion of cases is far greater than among normal non-breeding females.
4. The number of cases in which the anti-pig hemolysin is present tends in males to increase with the appearance of lung infection.

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STUDIES ON ABSORPTION FROM SEROUS CAVITIES

III. THE EFFECT OF DEXTROSE UPON THE PERITONEAL MESOTHELIUM

R. S. CUNNINGHAM

From the Anatomical Laboratory of the Johns Hopkins University

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It has long been known that absorption from the peritoneal cavity takes place rapidly and effectively. The absorption of solutions and particulate matter has been studied by many investigators, but very little effort has been made to use the peritoneal cavity as a route by which therapeutic agents might be administered. The basis of practical intraperitoneal therapeusis must be founded upon three general factors: *a*, the physiological and toxicological effects on the organism of substances administered by this route; *b*, the variations upon tissue resistance caused by the administration of any substance; and *c*, the direct effect upon the mesothelium. The determination of the effect of any substance upon the organism must be carefully controlled for each method of administration, as it is well known that there is a great variation in the effect of the same substance given by different routes. Such methods need not be discussed here, since they are in constant and practical use. In the peritoneal cavity the effect upon the resistance of tissues which are directly exposed to the first shock of the administration should be considered with very great care.

Blackfan and Maxcy (1) have reported the use of the peritoneal route for the administration of physiological saline to patients requiring additional fluids, with much success and no apparent ill effects. In their report they also give animal experiments in which they found rapid absorption of the saline; the peritoneum remaining smooth and glistening, no adhesions forming, and no abrasions of the viscera taking place. They have also shown that the introduction of salt solution is attended with no risk of infection if reasonable surgical precautions are taken, but they have not controlled their experiments by a study of the comparative resistance of normal animals, with animals which have been subjected to the intraperitoneal injection of repeated doses of physiological saline. This is obviously easy to determine with regard to any given substance, and any given exposure to that substance.

There has heretofore been no method for an early and certain diagnosis of injuries, or at least changes in the mesothelium. The formation of adhesions and an aseptic inflammation could of course be considered as definite criteria, but these are at best crude methods, and only indicate comparatively late stages of change in the mesothelium. It is now possible to estimate the effect of any given substance since it has been determined that definite morphological changes in the mesothelium can be produced by the repeated administration of certain solutions and suspensions. These changes have been produced by the introduction of particulate matter, laked blood and soluble starch. A preliminary note on these experiments has been published (Cunningham (2)); the detailed report has not been published but is in preparation. These findings seem to offer a possible criterion for the comparison of the effect which any substance will have upon the peritoneal mesothelium.

Dextrose was selected for study in this connection because, if it could be administered via the peritoneum without injurious effects, selection of that route would eliminate many difficulties incident to administration in other ways. The purpose of this contribution is not directed to the settlement of the action and fate of dextrose in the circulation, or to the reaction of the organism to this substance, but simply to the effect which it might have upon the mesothelium of the peritoneal cavity.

Technique and material. Rats were used for these experiments. The animals were anesthetised with ether, the abdomen shaved and washed with alcohol, and then a drop of tincture of iodine applied. The dextrose solution was introduced by means of a 10 cc. record syringe, a fresh needle being used for each animal. The syringe and needles were sterilized by boiling. The dextrose, Merck's "pure," was prepared by dissolving in freshly distilled water and sterilizing in the Arnold for one hour on each of two successive days. The solutions were never sterilized under pressure, because this usually caused the breaking down of the dextrose molecule. Cultures of these solutions made at the time of administration were uniformly sterile.

Two series of animals are to be reported at this time. The first consisted of twelve rats, each of which received 10 cc. of a 10 per cent solution of dextrose; seven of these were killed at two, four, six, eight, ten, twelve and fourteen hours respectively after the injection. The other five were killed at intervals between ten and fourteen hours; this was done in order to determine more exactly the time at which the fluid had been entirely absorbed. At autopsy cultures were taken, and the fluid in the peritoneal cavity was removed with a pipette; by this means practically all the fluid present could be recovered. Quantitative determinations of the sugar content were not done, because the actual rate of absorption of the sugar was not required for the present purpose. This will be referred to later. Tissue was fixed in Bouin's fluid, and prepared for section in the usual manner.

The second series consisted of twelve rats which received 10 cc. of a 10 per cent solution of dextrose every twenty-four hours for fourteen days. Six of these were killed at two, four, six, eight, ten and twelve hours respectively after the last injection. Three were killed at short intervals after twelve hours, in order to determine the end point of absorption as in the first series. The other three were kept as controls, and will be referred to later.

Experimental results. Figure 1 represents the amount of fluid found in the peritoneal cavities of the animals in the first series, while figure 2 is a similar curve for the second series. From these curves it is evident that the absorption of dextrose from the peritoneal cavity is not impaired by repeated doses of this solution, but the differences between the two curves are not great enough to establish conclusively that the exposure increases the ability of the peritoneum to absorb.

Autopsies on animals of series I revealed no changes in the peritoneal cavity, while examination of those in series II showed a slight brownish tint in the *taches laiteuses* of the omenta; otherwise they were entirely normal. No adhesions were found in any of the animals and the folds of the omenta were normal and free in every case. Usually three or four points of injection were visible as red areas about 1 mm. in diameter on the anterior abdominal wall; those from earlier punctures were almost entirely healed. Cultures taken at autopsy were sterile.

On histological examination of tissue from all the animals in the first series, the serous mesothelium appeared everywhere entirely normal and no changes were found in the *taches laiteuses*. On the other hand in the animals in the second series, which had received repeated doses of the dextrose solution, the mesothelium of the diaphragm, the spleen, the omentum, and in one case even the body wall, showed quite decided changes. The diaphragm and spleen always showed the most marked changes, which varied very little in the animals of the group, but the changes in the mesothelium of the omentum were much more variable, even in the same animal, while the mesothelial cells of the body wall showed slight increase in size in only one animal. This distribution of changes corresponded very closely to that found after the use of laked blood, and was most interesting in that the diaphragm and spleen were always most markedly affected, the omentum somewhat, while the intestines, the liver, and in most cases the body wall, showed little or no change.

The mesothelial cells of the diaphragm and spleen showed everywhere a definite increase in size, the nuclei having become rounded and vesicular, and the cytoplasm increasing considerably in thickness. The borders of the cells were often withdrawn from the neighboring cells, so that a small area of the subjacent connective tissue seemed to be uncovered. Here and there, especially over the diaphragm, a few cells had become almost separated from the underlying structures and were attached by only a small pedicle. Areas scattered over the diaphragm, which represent the space normally occupied by two or three cells, were bare; the cells evidently having desquamated. In the splenic mesothelium desquamation had not taken place to any great extent, although an occasional cell area was bare. Perhaps the most remarkable fact noticed was that, in the mesothelium of the diaphragm especially, but also in a less noticeable degree in the case of the spleen, many of the cells which had rounded up and increased in size were undergoing division. Mitotic figures could often be seen in several stages. In one section three figures were found in the area covered by the oil immersion lens. The cytoplasm of the mesothelial cells became much more basophilic as they rounded up and increased in size, but in no case could any granules in the cytoplasm be detected. Pycnotic nuclei were

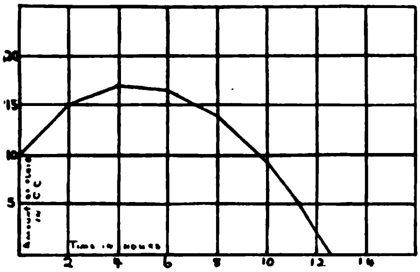

Fig. 1. Curve showing amount of fluid in peritoneal cavities of normal rats after introduction of 10 cc. of a 10 per cent solution of dextrose.

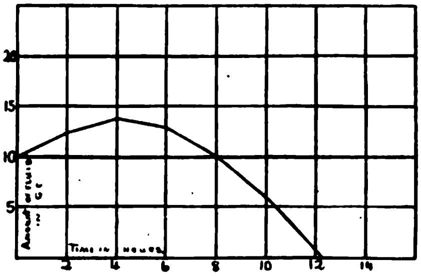

Fig. 2. Curve showing amount of fluid in peritoneal cavities of rats which had received fourteen injections of 10 cc. of a 10 per cent solution of dextrose.

never seen in any of these enlarged cells. One other interesting observation must be recorded; these large cells when carefully studied under very high magnification invariably presented a surface which was covered with fine projections simulating in appearance the cilia of normally ciliated cells. They differed from true cilia in that they varied considerably in length and width, and were somewhat irregular.

After examination of sections had demonstrated that dextrose produced such definite changes, other experiments were performed in order to determine how rapidly these changes occurred. Rats were given 10 cc. of dextrose solution and killed after one, two, three, four and five days respectively. After twenty-four hours no change was noticed. After forty-eight hours the only changes were a very slight thickening of the cell-bodies and a delicate irregularity of the peritoneal surface of the mesothelium, which suggested that the cells were beginning to form the cilia-like projections described above. After three or four days the cells had increased in size, and had become somewhat cuboidal in shape; while here and there some of them had begun to round up and the projections had increased. After the fifth injection the cells had rounded up and the free surfaces were covered with the characteristic cilia-like formations, but no evidence of any separation or desquamation was apparent.

Sections of the omenta of animals in the second series showed that the brown coloration mentioned earlier was due to a few fine granules of brown material in the cells of the *taches laiteuses*. These granules were very few in number and were scattered quite widely. There was no apparent change on careful comparison of sections of normal omentum with those of the rats in series II except for these few fine granules. Sections of anterior mediastinal lymph glands were examined to determine whether or not there was sufficient granular material in the sugar solution to have produced the changes noted in the mesothelium; but only an occasional granule could be found in any of the cells, which are usually loaded with them, when particulate matter has been absorbed from the peritoneal cavity.

In order to determine the effect of rest after exposure to dextrose the three rats, saved as controls from the series of animals which had received fourteen doses, were killed six, eight and ten days after the last dose was given. In all of these, sections demonstrated that the mesothelium was everywhere absolutely normal in appearance. From this it was evident that recovery had taken place completely in six days.

DISCUSSION

These experiments do not settle the question of the feasibility of using the peritoneal cavity as a route for the administration of dextrose; but they furnish certain evidence which is of value, both in regard to this practical application and to the general reactions of the peritoneal mesothelium. The experimental results which are favorable for the use of this method are: the general effect upon the animals, the nature of the curve of absorption, the rapid recovery of the mesothelium, and the absence of adhesions. But the changes in the mesothelial cells can not be considered as directly favorable, and may even prove a definite contraindication.

The animals suffered no ill effects as far as could be judged from their activity, general appearance and weight. While the rats were not weighed every day, their weights were taken before the first injection, and occasionally thereafter; none were found to have lost weight, while several actually gained a little. In studying the two curves it is evident that the length of time required for the complete absorption of the dextrose solution was about the same in both series of animals; but the increase in fluid, soon after the injection, was considerably greater in the first than in the second. From these observations it is evident that the continued exposure of the surfaces of the peritoneum did not produce sufficient injury or change to decrease their ability to absorb this particular solution.

In addition to this conclusion these curves suggest the discussion of certain general features of absorption, concerning which we have very little conclusive evidence. Immediately after the injection of the sugar solution the total amount of fluid in the peritoneal cavity increases, and this can only be at the expense of the water in the tissues and blood stream. At the same time we may conclude that the sugar is passing out of the peritoneal cavity into the blood stream and tissue spaces, although this has not been established in these experiments by quantitative examination of the fluid.

In short an equilibrium is being established by osmosis and diffusion between the two systems: the sugar solution which has been introduced into the peritoneal cavity, and the contents of blood vessels and tissue spaces. In considering the absorption of the solution after the equilibrium has been reached, which is indicated by the highest points of the curves, it seems evident that some other factor besides osmosis and diffusion must be involved. The most plausible explanation for this remarkable absorption, which takes place against the concentration gradient, seems to be that the sugar molecules are drawn over into the blood stream and tissue spaces by means of some difference in electrical potential; and this increase in concentration of the dextrose in the blood forces the water out of the peritoneal cavity. The evidence obtained from these experiments is insufficient to establish any hypothesis regarding the nature of this exchange.

The nature of the change in the mesothelium has not yet been established, and upon this depends whether or not the reaction of these cells can be considered as a definite contraindication to the administration of dextrose via the peritoneal cavity. If the change is fundamentally an injury, if the desquamating cells are badly injured or dying, and if those remaining are unable to recover the denuded areas, then adhesions are likely to form and inflammatory processes to develop, if any organism should obtain access to the peritoneum. On the other hand if the change in these cells is the result of stimulation, if the cells are actively proliferating, if those cells which do separate are viable and continue life as free macrophages, and if the remaining cells are capable of renewing the denuded spaces so that only a few cell areas will be empty at any time, then adhesions should not develop and the protection against adventitious infection would seem likely to be as great as ever. Theoretical possibilities must include combinations of stimulation and injury, in which case any application would depend upon the ratio between the two.

These questions are most difficult to settle experimentally; the evidence obtained from these experiments tends to support the suggestion that the changes are due to stimulation. The mesothelium, after exposure to repeated doses of a 10 per cent solution of dextrose during a period of fourteen days, does not show denuded areas larger than the space normally occupied by two or three cells, there is no evidence of injury or death, but rather of active proliferation in these cells, no adhesions have occurred, and recovery, either by the return to normal of the cells which have rounded up or their replacement by other cells, is complete in six days.

The distribution of this change over diaphragm, spleen and omentum, while the remainder of the peritoneal surfaces is comparatively normal, would indicate that there is some special differentiation of the mesothelium in these regions. And it is quite possible that the explanation of the observed reaction should be sought in this differentiation rather than in some injurious effect produced on the cells by the dextrose.

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THE EFFECT ON THE COMPOSITION OF THE BLOOD OF MAINTAINING AN INCREASED BLOOD VOLUME BY THE INTRAVENOUS INJECTION OF A HYPERTONIC SOLUTION OF GUM ACACIA AND GLUCOSE IN NORMAL, ASPHYXIATED AND SHOCKED DOGS

H. L. WHITE AND JOSEPH ERLANGER

From the Physiological Department of Washington University, St. Louis

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The present work was undertaken with the idea of investigating the effect upon the composition of the blood of producing and then maintaining for some hours an expansion of the blood volume through the intravenous injection of a hypertonic crystalloid and colloid in combined solution, in the hope of throwing some light on the mechanism of the processes involved. The solution injected consisted of 18 per cent glucose and 25 per cent gum acacia and is the one that has been found useful in the treatment of traumatic shock (1). Studies of the blood changes produced were carried out under conditions which were varied in a way that might change the permeability of the vessel walls to the tissue fluids and their various constituents.

The literature contains many references to studies, more or less complete, of the blood changes following the intravenous injection of crystalloids, glucose, NaCl, Na_2SO_4 and urea being the substances most commonly used. Brasol (2) found that following intravenous injection of a hypertonic glucose solution the blood was greatly diluted in 2 minutes but that the blood volume had returned to normal within 2 hours. He found that there was no relation between the amount of glucose injected and the per cent of glucose found in the blood 2 minutes later, that the blood sugar was normal 2 hours after the injection, that part of the sugar which leaves the blood can be found in the tissues, but part cannot be found and is perhaps changed to glycogen, lactic acid or "undergoes some other chemical metamorphosis," and that the absolute quantity of protein in the serum remains unchanged. He concludes that the effects produced are purely the results of the increased intravascular osmotic tension due to the injected glucose. Klickowicz (3) obtained similar results as regards blood volume with strong solutions of Na_2SO_4. Leathes (4) finds that the "increase in the volume of the blood caused by the injection of 5 grams of dextrose per kilo is enormous and quite out of proportion to that caused by the volume of the injection. This increase takes place with remarkable rapidity; the volume of the blood is nearly doubled by the time the injection is finished." Gasser and Erlanger (5) followed the blood volume after intravenous injection of 5 cc. of 18 per cent glucose solution per kilo body weight, the injection being made as rapidly as possible. They found that the maximum dilution was attained within 0.5 to 2 minutes and that the blood then began to concentrate, rapidly at first, and then more slowly, and became normal within 5 to 45 minutes. Starling (6) finds hydremic plethora following glucose injection and that the distended vessels begin at once to unload the excess of water. Paton (7) finds that when 4 grams of carbohydrate in 10 cc. of water per kilo body weight are injected intravenously only a small per cent of the amount injected can be recovered immediately from the blood, the time from the beginning of the injection to the end of collecting the sample being 90 to 100 seconds. Hamburger (8) finds that in the horse an intravenous injection of 7 liters of 5 per cent Na_2SO_4 causes a marked dilution of blood serum in respect to its chloride and protein content, a return to normal being accomplished very quickly, within 2 hours. Practically the same effect on protein content of serum follows the injection of 5 liters of 0.5 per cent Na_2SO_4 solution, while the chlorides are diluted as with a hypertonic solution but do not return to normal. The blood volume was not followed in these cases, so no conclusions can be drawn as to whether the return to normal was due to a passage of water out of the blood stream or an entrance of solids into the blood stream. Fisher and Wishart (9) found that blood dilutes as a result of alimentary hyperglycemia. Magnus (10) obtains varying results in his studies of changes in blood composition following the intravenous injection of NaCl solutions, the results depending upon the osmotic tension of the injected solution, although consistent results were not obtained with hypertonic solutions.

The common feature of the experiments discussed above is that the substances injected were crystalloids and, with the exception of those of Fisher and Wishart, the injection was rapid; and the increase in blood volume, with hypertonic solutions, always is very rapid in appearance and of short duration. Woodyatt, Sansum and Wilder (11) injected glucose intravenously over long periods of time at a uniform rate and determined the tolerance rate as 0.8 to 0.9 gram per kilo per hour. Erlanger and Woodyatt (12) injected glucose intravenously at uniform rates varying between 0.57 and 4 grams per kilo per hour for from 20 to 60 minutes into anesthetized animals in shock. The pulse amplitude was uniformly markedly increased, indicating a condition of plethora. A subtolerant dose was as effective as injections made at more rapid rates. In a normal animal an injection lasting 30 minutes at the rate of 1.78 gram per kilo per hour raised arterial pressure only about 5 mm. Hg. but the pulse amplitude increased quite appreciably. The fact that a considerable increase in blood volume is accompanied by only a slight rise in arterial pressure is presumably due to diminution in viscosity of the blood and to vasomotor accommodation. While the blood volume was not followed in these cases it presumably soon fell after cessation of injection to its pre-injection state.

With regard to the entrance of protein into the circulation, Morawitz (13) found restoration of proteins most active in the first few hours after severe hemorrhage. Whipple and co-workers (14) find that after severe hemorrhage the regeneration of plasma proteins is much more rapid on a liberal high protein diet than on a liberal bread and milk diet, and least rapid on fasting. They infer from this that there is an actual new formation of protein and make no mention of the possibility of some of the protein being drawn in from the tissue fluids. They also find that "after the initial depletion of serum proteins the body can almost always regenerate 1 per cent of the total protein during the first 24 hours following the plasmapharesis. This figure is remarkably constant and does not seem to be influenced by diet or fasting . . . . or the amount of shock. We may perhaps look upon this as the maximum effort of the body to replace these essential serum proteins, an effort which surely depends upon the body protein as it is present in fasting experiments. It will be the same whether there is a marked breakdown of host protein, as evidenced by great increase in urinary nitrogen, or whether this tissue autolysis is minimal." That they do not regard this maximum effort as accomplished by the passage of protein directly into the blood from the tissues but rather as a true rebuilding is shown by their further statement that it "probably represents the absolute maximum production under the greatest stimulus." They present further evidence that the restoration of plasma proteins after hemorrhage is due to a new formation of proteins and that the liver is the chief seat of this process in that restoration is greatly hindered by phosphorus and chloroform poisoning and by an Eck fistula.

A rather careful review of the literature has failed to disclose any references to studies of the body fluids made during a period of prolonged increase of blood volume due to the injection of hypertonic solutions. It is evident that only two means are at our disposal for maintaining an increased blood volume for a comparatively long period, several hours for instance, with fluids other than blood or blood plasma. One is by a continuous injection of a hypertonic or isotonic solution of crystalloids at a rate more rapid than the rate of the blood's fluid loss. In this case the large amount of fluid injected would so dilute the blood that no conclusions could be drawn as to the interchange of water and solutes between tissue spaces and blood stream. The other method is to inject a small volume of a strongly hypertonic solution which will not only draw fluid into the blood stream but hold it there for some time. To accomplish this result a solution containing both crystalloid and colloid is necessary (15). So far as we know the present work represents the first effort made to study changes in the composition of the blood induced in this way.

PROCEDURE

Dogs were used in all experiments. The animals were not prepared by controlling their intake of food or water previous to the experiment. Most of them were fed and watered on the morning of the experiment. In the first seven cases the animal was given 1 grain of morphine an hour before starting the operation; in the case of dog 8, 2 grains were given by mistake. In the remainder of the cases the morphine was omitted because of its disturbing effect on the blood and urinary sugar. In the first four cases strict surgical asepsis was observed throughout the experiment; in the remainder these precautions were relaxed, although the fluid injected and the injecting apparatus were sterile. No differences in results due to these technical differences were observed. Three sets of experiments were performed; the first, a series of nine experiments, on normal dogs; the second, a series of two, on asphyxiated dogs; the third, a series of three, on dogs in shock. The procedure and data of each series are presented separately.

The *procedure in the first series* was to anesthetize the dog with ether, immediately draw a "standard" sample of blood from the femoral artery, then to start the injection into the femoral vein. The dose in all cases was 5 cc. per kilo per hour of an 18 per cent glucose and 25 per cent gum acacia solution, the injection lasting 1 hour and proceeding at a uniform rate. Immediately at the termination of the injection another sample of blood was taken and the dog put back into its cage. Subsequent samples were taken at approximately 2-hour intervals until five samples were obtained. Little or no anesthetic was required while drawing the last three samples. On each sample determinations were made of hemoglobin (16), plasma chlorides (17), plasma total nitrogen and plasma non-protein nitrogen (18), and plasma sugar (18). The nitrogen and chloride determinations were made in duplicate. Direct Nesslerization was at first attempted for both the total and the non-protein nitrogen determinations, appropriate dilutions being made, but it was found that the presence of the large amount of carbohydrate in the samples rendered digestion with the prescribed digestion mixture so difficult that indirect Nesslerization had to be resorted to. In the latter cases of the series the total nitrogen determinations were made by the macro-Kjeldahl method since it was felt that the micro-Kjeldahl with indirect Nesslerization was not sufficienty trustworthy. Figures given in the tables under the heading "total N" are protein nitrogen figures and are obtained by subtracting the non-protein nitrogen figure from the total nitrogen figure as determined by the Kjeldahl. Several dilutions of the filtrate for plasma sugar determinations were made, the dilution being accepted whose reading was closest to 20, the standard being set at 20 on the Dubosq or Bock-Benedict colorimeter. Freezing point determinations were attempted in the first experiment, working with 1.5 cc. of hirudinized plasma in a special tube in the Beckmann apparatus, but it was found that consistent results could not be obtained with this amount of plasma and these determinations were discontinued as it seemed inadvisable to take more blood. In lieu of direct determinations the crystalloid osmotic pressure of the plasma has been roughly estimated by adding the osmotic pressures exerted by the NaCl and glucose present. The calculations were made from published tables of direct osmotic pressure determinations and of freezing point determinations. By using various schemes for utilizing every available drop of plasma it was possible to make all determinations with 13 cc. of blood drawn at each sample. Allowance for blood drawn as samples has been made in the calculations. Dogs 8 and 9 were bled 15 per cent of their blood volume at the time of taking the first sample. In the last few cases the urine was also followed, the bladder being emptied as completely as possible with a small coudé catheter at the time of drawing the blood samples. The results of the first series are given in table 1.

The *procedure in the second series* was to anesthetize the dog with ether (morphine being omitted in these and subsequent experiments), insert a tracheal cannula, draw a standard sample of blood from the femoral artery and empty the bladder. The animal was then made to rebreathe air in a large spirometer until the CO_2 tension of the air in it mounted to about 30 mm. Hg. as determined with Marriott's (19) tubes on samples drawn from the spirometer. The injection was then started, the CO_2 tension being kept around 30 to 35 mm. Hg., fresh air being admitted at intervals when the dog became markedly dyspneic. Dyspnea was moderate to marked throughout the injection. This procedure was introduced in an effort to discover whether asphyxia altered the permeability of the vessel walls. At the conclusion of the injection the second sample of blood and urine was drawn, rebreathing discontinued, the animal put back into its cage and subsequent samples taken as in the first series. The results of the second series are given in table 2.

In the case of dog 10 the injection rate was not accurately timed, at first. Twenty-five cubic centimeters were run in during the first 20 minutes. The injection was then stopped for 10 minutes, the remainder of the injection then proceeding at the proper rate. When the last sample was drawn from dog 10 he struggled quite vigorously and it was necessary to anesthetize him deeply, which is not usually necessary for the last three samples. This anesthesia probably accounts for the high blood sugar value in the last sample of dog 10.

The *procedure in the third series* was to anesthetize the animal, insert a tracheal cannula and connect a manometer with a femoral artery, draw a standard sample and then put the animal into a condition of shock. The condition induced was the so-called mechanical shock of Janeway and Jackson (20), which they produced by temporary partial occlusion of the vena cava regulated so as to keep the blood pressure at 30 to 40 mm. Hg. for 2 hours. The method of obstructing the cava used in the present work was one described by Erlanger and Gasser (21), a clamp being used by which graded compression can be exerted upon the cava between the liver and the diaphragm through a small abdominal incision. The mean arterial pressure was kept at about 40 mm. Hg. for 120 to 135 minutes. At the end of this time the clamp was removed, the second sample drawn, the injection given, a third sample then taken, and the dog kept as long as he would live, samples being drawn at about 2- or 2½-hour intervals. Very little anesthetic was required to keep the dog quiet after shock had been produced. This procedure was employed in an endeavor to ascertain whether the permeability of the vessel walls is altered in shock.

The results of the third series are given in table 3 and in the brief case histories.

DISCUSSION OF RESULTS

Series I. Normal Dogs

1. Blood volume. It is evident that in all cases there is a marked immediate increase in blood volume, amounting to 11 to 16 per cent, which usually slowly returns toward normal. Some slight increase in volume, about 2 per cent, usually persists even after 6 or 7 hours. The combined action of the glucose and acacia in effecting changes in blood volume is interesting. The early high intravascular osmotic tension which causes the rapid increase in volume is due principally to the glucose. The glucose, however, rapidly passes out into the tissue spaces (see plasma sugar figures) but through the influence of the gum acacia the water very slowly leaves the vessels. The persistent increase of blood volume, 2 or more per cent, is less than the amount of water which must be added to the amount of acacia presumably remaining in the blood (22) in order to make it isosmotic to the plasma proteins. It will be noticed, however, that in every case but one, dog 3, where the figures are the same, the total amount of plasma proteins at the close of the experiment is less than that at the start. Part of the injected acacia, then, presumably is taking the place of the removed plasma protein in holding water, and only the remainder can be employed in holding an excess of water above normal blood volume. Quantitative determinations of acacia in the blood would be necessary in order to ascertain just how much acacia is available for maintaining an increased blood volume. Any fraction of the persisting increase in intravascular osmotic tension which might be due to the glucose could not play any part in maintaining the increase in blood volume, since glucose now exists in the tissue spaces in equal concentration, having passed out from the blood stream, and is exerting an equal attraction there. In fact, the blood sugar figures show that the excess of glucose has been entirely removed from the blood stream, and presumably from the tissue fluids, inside of 2 hours. The fate of the glucose will be discussed later.

TABLE 1

Data of series 1, normal dogs

DOG	SAMPLE	TIME	BLOOD VOLUME	PLASMA CHLOR.	SUGAR	N. P. N.	PROTEIN N				URINE		
							Remaining	Withdrawn	Total	Per 100 cc. plasma	Amount	Sugar	Sugar
		hours	*per cent*	*grams per 100 cc.*	*grams per 100 cc.*	*mgms. per 100 cc.*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*	*grams*
1	1		100	0.631									
	2	0	118	0.607									
	3	1 5′	112	0.619									
	4	3 15′	105	0.628									
	5	5 25′	106	0.629									
2	1		100	0.612	0.196	32							
	2	0	111	0.581	0.341	31							
	3	2	108	0.602	0.200	31							
	4	4	105	0.608	0.125	32							
	5	6 30′	103	0.610	0.127	31							
3	1		100	0.605	0.192	26	5.05		5.05	0.805			
	2	0	116	0.562	0.247	27	5.41	0.06	5.47	0.685			
	3	2	115	0.582	0.219	26	5.35	0.12	5.46	0.685			
	4	4 10′	113	0.589	0.127	25	5.21	0.18	5.39	0.685			
	5	6 15′	112	0.600	0.133	26	5.06	0.24	5.30	0.675			
4	1		100	0.595	0.282	32	4.53		4.53	0.837			
	2	0	114	0.537	0.564	33	4.11	0.06	4.17	0.622			
	3	2 20′	109	0.558	0.182	32	4.21	0.12	4.33	0.680			
	4	4 30′	105	0.572	0.182	33	4.31	0.18	4.49	0.742			
	5	7 25′	102	0.586	0.170	33	4.40	0.24	4.64	0.794			
5	1		100	0.579	0.232	25	3.94		3.94	0.720			
	2	0	116	0.533	0.381	25	no det.	0.06					
	3	2 5′	113	0.554	0.178	26	3.79	0.12	3.91	0.572	6	4.8	0.29
	4	4 15′	109	0.570	0.167	25	3.89	0.18	4.07	0.623	8	6.0	0.48
	5	6 40′	102	0.574	0.172	26	3.50	0.24	3.74‡	0.624	35	3.2	1.12
	Total										49		1.80

6	1			100	0.635	0.222	33	6.46		6.46	0.852			
	2	0		113	0.601	0.500	32	6.08	0.07	6.15	0.660			
	3	2		112	0.618	0.217	33	6.09	0.14	6.25	0.674			
	4	4	25′	106	0.632	0.215	33	6.10	0.21	6.31	0.727			
	5	7	40′	101	0.635	0.204	32	5.96	0.28	6.24	0.772	245		5.6
Total												245	2.3	5.6
7	1			100	0.582	0.308	31	4.35		4.35	0.833			
	2	0		111	0.560	0.541	30	4.54	0.07	4.61	0.744			
	3	2	10′	106	0.568	0.342	31	4.42	0.14	4.56	0.760			
	4	4	20′	102	0.574	0.250	31	4.16	0.21	4.37	0.774			
	5	7		98	0.579	0.185	30	4.06	0.28	4.34	0.800	20	2.3	0.46
Total												20		0.46
8*	1			100	0.642	0.400	28	10.01		10.01	1.14	21	1.7	0.35
	2	0		99	0.610	0.606	27	8.72	1.555	10.27	1.03	108	7.1	7.7
	3	2		95	0.630	0.286	28	8.55	1.64	10.19	1.062	80	6.4	5.1
	4	4	10′	89	0.636	0.234	27	7.88	1.73	9.61	1.091	28	4.3	0.55
	5	6	20′	86	0.640	0.220	27	7.54	1.82	9.36	1.115	55	1.0	0.55
Total after injection												271		14.55
9†	1			100		0.133						200		0
	2	0		106		0.292						10		f. t.
	3	2		97		0.163						14		0.17
	4	4		94		0.133						20		tr.
	5	6	10′	93		0.135						42		0
Total after injection												86		0.17+

* Dog 8 bled 220 cc. at 1st sample.

† Dog 9 bled 160 cc. at 1st sample.

‡ Evidently some mistake in last determination.

The rather wide variations in the rate of the falling off from the initial increase in blood volume possibly are to be attributed to variations in vasomotor accommodation and in the preexisting water content of the tissue spaces. Scott (23) finds the hemoglobin content increased immediately by a rise in blood pressure produced by various procedures, and lowered by a fall in pressure. He cites evidence indicating that the hemoglobin method gives an accurate picture of relative blood volume; and, as he says, "these results can only be explained by increased pressure forcing fluid out of the blood to the tissue spaces and the passage of fluid back from the tissue spaces to the blood when the pressure is lowered." He did not determine the nature of the interchanged fluid.

At the time of taking the first sample dog 8 was bled approximately 15 per cent of his calculated blood volume, 15 per cent having been about the average initial increase in blood volume following the injection. This was done in order to ascertain whether the interchange of liquids and solids between the blood and the tissues is modified when the plethora that is induced by the injection of a hypertonic solution is prevented by previous hemorrhage. Blood volume figures indicate that the liquid interchange is about as in unbled animals. The question of the departure of the behavior of the solids in this animal from the behavior of the interchanges of solids in animals without previous hemorrhage will be considered below. In the case of dog 9 the 15 per cent depletion of the blood is more than made up by the time of the termination of the injection (blood volume raised to 106 per cent of normal); this volume falls in 2 hours to 97 per cent, then very slowly to reach 93 per cent at the end of 7 hours. The gum acacia is in all probability filling the place of the removed plasma proteins in holding water.

2. Blood composition. The main point of interest is, What is the nature of the fluid drawn into the blood stream and how does its composition change subsequently? A satisfactory answer to these questions should serve to elucidate the processes determining the interchanges.

a. Protein. Any conclusion as to the passage of protein into or out of the blood stream must be based on calculations of the absolute amount of plasma protein in the entire blood stream. If, for instance, it should be found that the curve representing the percentage of plasma protein ran parallel to the curve representing the reciprocal of blood volume, this would not mean that the absolute protein content of the plasma was unchanged, since practically all of the fluid drawn into the blood stream enters the plasma and an increase of 15 per cent in blood volume would mean an increase of over 23 per cent in plasma volume. The method used here of calculating absolute amounts of plasma protein is essentially that used by Magnus (10), some changes in the method being introduced in order to allow for the hemoglobin withdrawn in the samples. It assumes that the blood is 9 per cent of the body weight (24), (25), (26), this figure being used instead of 7 per cent used by Magnus, that the blood is 64 per cent plasma by volume and that all the water drawn into the blood stream enters the plasma. While none of these three assumptions is strictly correct, the first and third are very nearly so and variations in the second will not involve gross errors since the calculations for the additional samples of any one animal are based upon the plasma volume figure obtained in the calculation of the standard blood of that animal. A sample calculation may be given for purposes of illustration.

Sample calculation. Dog 7. Body weight, 9.1 kilos

HEMOGLOBIN				TOTAL NITROGEN*	
Sample number	Reading	Volume per cent uncorrected	Volume per cent corrected	Sample number	Grams N per 100 cc.
1	20.0	100	100	1	0.83
2	22.6	113	111	2	0.74
3	22.0	110	107	3	0.76
4	21.4	107	102	4	0.77
5	21.0	105	98	5	0.80

* The terms "N" or "Total N" refer to protein N, i.e., the non-protein N has been subtracted from the Kjeldahl figure.

Method of correcting blood volume for hemoglobin withdrawn. For each sample 13 cc. of blood are removed. This is 1.6 per cent of the blood volume of this animal, or 1.6 per cent of 819, which is 0.09 × 9100. Since, after the first sample, only 98.4 per cent of the original amount of hemoglobin remains in the blood stream the increase in volume of the blood is only 98.4 per cent of that indicated by the blood's dilution as shown by the hemoglobin readings in the colorimeter. To illustrate, assume that 5 per cent of blood and therefore of hemoglobin is withdrawn from a normal dog, assume that the normal or standard sample reads 20 in the colorimeter, as in our case, and assume further that a second sample drawn subsequent to the withdrawal of 5 per cent blood volume reads 21 in the colorimeter. If we failed to consider the previous loss of the 5 per cent hemoglobin we would infer from this reading that the blood volume had increased 5 per cent, was now 105 per cent of the normal. What has actually happened, however, is that the blood volume has been replaced just to its original state, the diluent being of course hemoglobin-free, so that each unit volume of blood now contains only 95 per cent of its normal amount of hemoglobin. To find the true blood volume we should take 95 per cent of 105 or 100 per cent, in round numbers. To find the true blood volume in any case, therefore, we should multiply the apparent blood volume by the percentage figure of hemoglobin remaining in the blood stream at the time of drawing the sample.

Upon the basis of this treatment of the data we find that in dog 7 the blood volume at the time of drawing the last sample is actually slightly decreased rather than increased, as the hemoglobin readings would seem to indicate, i.e., the decrease in the percentage of hemoglobin in the last sample is not great enough to account for all the hemoglobin removed. We must assume, therefore, that the blood volume has fallen slightly. The decrease, however, is not as great as the volume lost by hemorrhage. This means that some of the fluid drawn in has stayed in but not quite enough to equal the amount lost by hemorrhage. In all the other normal animals the final blood volume even after correction for the withdrawal of blood was slightly above the initial or normal volume.

Using the corrected blood volume figures we may proceed with the *method of calculating total plasma nitrogen.* It may be noted here that the figures for blood volume per cent given in all the tables have been corrected for the amount of blood lost according to the method outlined above.

0.09 × 9100 = 819 cc., blood volume at beginning, blood 1.

0.64 × 819 = 524 cc., plasma 1.

0.83 × 5.24 = 4.35 grams N in plasma 1.

Blood 2 is 111 per cent of blood 1, by volume. The 111 per cent increase has been in the plasma. 0.11 × 819 = 90 cc. increase.

Plasma 1 = 524 cc. 524 + 90 = 614 cc., plasma 2.

0.74 × 6.14 = 4.54 grams N in plasma 2.

Blood 3 is 107 per cent of blood 1. The 7 per cent increase has been in the plasma. 0.07 × 819 = 57 cc. 524 + 57 = 581 cc., plasma 3.

0.76 × 5.81 = 4.42 grams N in plasma 3.

By the same method we get 0.77 × 5.40 = 4.16 grams N in plasma 4.

0.80 × 5.08 = 4.06 grams N in plasma 5.

These figures indicate that by the time sample 5 was drawn protein had passed out of the blood stream, i.e., less protein is in the plasma than was there at the beginning. If, however, we add to each plasma N figure the amount of plasma N taken out in that sample and in preceding samples we get the following figures: The amount of N withdrawn with each sample is 0.64 × 13 × 0.008 = 0.07 gm. N, 0.008 being taken as the average amount of N per cc. plasma in this animal. Variations in different samples are not significant in calculations on 13 cc. The effect is of course cumulative; 0.14 gm. will have been removed by the time the third sample is drawn, 0.21 gm. by the time of the fourth, etc.

SAMPLE NUMBER	PROTEIN N		
	N remaining	N withdrawn	Total protein N
	grams	*gram*	*grams*
1	4.35	0.0	4.35
2	4.54	0.07	4.61
3	4.42	0.14	4.56
4	4.16	0.21	4.37
5	4.06	0.28	4.34

These figures show that there is at first a slight increase in the amount of plasma protein but that by the time of the fifth sample this has disappeared, and that the final total plasma protein N is the same as the original figure if the amount withdrawn is added. Different animals show some variations from this behavior.

The figures obtained on the dogs without hemorrhage are not constant. All show that there is a marked decrease in the concentration of plasma protein accompanying the increased blood volume; but two cases, dogs 3 and 7, show that this decrease is not quite so great as the increase in plasma volume, i.e., a slight amount of protein enters the blood stream; while two others, dogs 4 and 6, indicate that a slight amount of protein passes out of the blood. Of these cases, however, the figures for dog 7 are the most reliable, since they were obtained by Kjeldahl determinations, using 2 cc. of plasma, while the others were obtained by indirect Nesslerization, working with 0.05 cc. of plasma. While the figures are not convincing, it appears that there is a slight increase in the absolute amount of plasma protein immediately after the injections. If the amounts of protein withdrawn in taking the samples are added to the amounts remaining we find a greater, though still quite slight, increase and that the amount of plasma protein tends to become constant in most cases at a level slightly above or about the same as the original one.

Scott (27) showed that dilution of the blood *in vitro* with isotonic Ringer's solution causes protein to pass from the blood cells into the plasma. He uses this observation to account for the increase in total plasma protein following hemorrhage or the injection of isotonic Ringer's solution (28). By inferring that slight variations from the proper proportions of the salts in the fluid added to the blood greatly inhibit this transfer of protein, he accounts for the greater increase in plasma protein after hemorrhage.

In order to determine whether or not the increase in total plasma protein which we found could be due to a passage of protein from blood cells to plasma the following experiment was performed. From an 8 kilo dog 140 cc. of blood were drawn into a vessel containing the minimum amount, 0.12 gm. per 100 cc. blood, of potassium oxalate that would prevent clotting. This was stirred and 45 cc. samples immediately put into each of three cylinders. All three were stirred continuously while to one was added 2.5 cc. of the gum-glucose solution at a constant rate over 40 minutes, to the second 2.5 cc. of gum-glucose solution + 5 cc. Ringer's solution with the calcium omitted; the third was merely stirred and used as a control. The second of the above mentioned procedures may be assumed to approximate the conditions obtaining in the intravenous injection *in vivo* of 5 cc. of the gum-glucose solution per kilo body weight or per 90 cc. blood, the 5 cc. Ringer's per 45 cc. blood being the average increase in blood volume over the amount of fluid injected. After the addition of the solutions was completed hematocrit determinations were made on each sample and Kjeldahl nitrogen determinations made on each plasma.

SAMPLE	HEMATOCRIT			PLASMA IN 45 CC. BLOOD	PROTEIN IN PLASMA
	Total	Cells	Plasma		
				cc.	*gram*
1 (45 cc. blood)	10.7	4.2	6.5	27.4	0.259
2 (45 cc. blood + 2.5 cc. g.g.)	11.0	4.1	6.9	28.2	0.260
3 (45 cc. blood + 2.5 cc. g.g. + 5 cc. Ringer's)	11.4	3.6	7.8	30.8	0.257

The results collected in the accompanying table show that there was no exchange of protein between cells and plasma. It seems fair, therefore, to eliminate the blood cells as a source of the increased plasma protein in our *in vivo* experiments.

Kjeldahl determinations were also made on the gum-glucose used in order to determine if it might be a source of N. It was found that 2 cc. of the solution is equivalent to 0.6 cc. tenth normal HCl. Expressed in percentage of N our determination shows 0.168 per cent N in the gum acacia or 0.042 per cent N in the solution injected. Rideal (29) finds gum acacia to contain only 0.031 to 0.082 per cent N. Other observers have reported varying percentages of N, depending upon the source of the gum. The form in which the N exists has never been determined, so far as we have been able to learn. The amount of the solution used for a 10 kilo dog, 50 cc., would contain only 0.021 gm. N, according to our determinations. Corpuscles and injected solution may therefore be disregarded as a source of the increase in plasma N. Our results lead us to the conclusion, therefore, that the injection of the acacia-glucose solution leads in some way to the entrance of protein into the circulation.

The changes in the percentage concentration of plasma protein following the injection are also of interest in that they indicate roughly the changes in the colloidal osmotic tension of the blood. It is seen that the concentration falls markedly immediately after the injection, considerably more than can be accounted for by the blood's dilution due to the injected fluid alone. Let us assume that each 5 cc. of the injected solution contains 4.5 cc. of water. Since 4.5 cc. of water are injected per kilo body weight or per 90 cc. of blood or per 58 cc. of plasma, the plasma is diluted 7.8 per cent by the water injected. The plasma protein percentage, however, invariably falls more than this, averaging about 15 per cent, showing that not only the fluid injected but the fluid drawn in from the tissues dilutes the plasma in respect to its protein content. The plasma protein content in the subsequent hours rises toward normal but never reaches it, this state of affairs co-existing with a slight persisting increase in blood volume. The assumption seems justified that part of the colloidal osmotic tension of the plasma is being supplied by the injected gum acacia.

Magnus reports two experiments with intravenous injection of concentrated (35 per cent) NaCl solution. In one case he finds the total plasma proteins diminished by 7 per cent by this procedure, in the other case increased by 5 per cent. These were on previously unbled dogs and the results are comparable to our results on previously unbled dogs.

Dog 8, *which was bled 15 per cent of his blood volume* before the injection, shows a decrease in total plasma protein at the second sample. When, however, the amount of plasma protein withdrawn by hemorrhage is added to the total amount in the blood at the time the second sample was drawn we find a slight increase in the plasma protein—some protein has passed in.

b. Sugar. It is seen that the initial blood sugar values in the first 8 cases are very high, 0.192 to 0.308 gm. per 100 cc. This is due to the well known action of morphine as a respiratory depressant, the imperfect respiration causing a hyperglycemia. At the close of the injection the figure is still higher, 0.247 to 0.564 gm. per 100 cc., due to the injected glucose. Within 2 hours the figure has usually fallen to or below the initial value. The sugar figures on dog 9 are much the most valuable since here the disturbing effect of the morphine is not present. The initial figure is 0.133, the slight increase above normal probably being due to the ether; the figure immediately at the termination of the injection is 0.292; 2 hours later, 0.163; 2 hours later, 0.133; and the last sample, 2 hours after this, contains 0.135 gm. These figures show that the sugar rapidly passes out of the blood, and is almost back to normal in 2 hours. The fate of the sugar will be considered later.

Since the increased blood volume persists for several hours after the blood sugar has returned to normal it must be concluded that gum acacia remains in the circulation and holds water there. The great difficulty of digestion for nitrogen determinations of all the samples taken subsequent to the injection, even those which had regained a normal blood sugar value, indicates that there was an abnormally large amount of carbonaceous material in the blood and confirms the view that gum acacia remains in the blood for some time.

c. Plasma chlorides. The plasma chlorides were followed because it was felt that their behavior under the conditions of the experiment would be typical of that of the inorganic crystalloids in general. The percentage concentration of plasma chlorides always falls in the second sample, but not in any way to the same extent that the blood is diluted; in fact, the percentage fall in concentration is not even as great as the percentage of dilution of plasma accomplished by the injected fluid. It has been seen above that the plasma is diluted about 7.8 per cent by the injected fluid. The percentage fall of plasma chloride concentration is less than this, averaging about 6 per cent. In other words, the fluid drawn into the blood stream carries with it chlorides in concentration equal to that of plasma and in addition to this inward filtration of chlorides an inward diffusion has started to supply chlorides for the injected fluid. This diffusion continues for several hours, the percentage of plasma chlorides steadily rising and becoming normal in 6 to 8 hours.

d. Non-protein nitrogen. It will be noted that the plasma N.P.N. concentration remains constant with variations in plasma volume, indicating that the vessel walls are more permeable to urea than to NaCl. The constant concentration of N.P.N. means that not only does the fluid drawn in have a N.P.N. concentration equal to that of plasma but also that the diffusion into the blood stream of the N.P.N. necessary to make up the injected fluid to the concentration of N.P.N. existing in plasma is very rapid, being completed by the time of completion of the injection. Urea's greater solubility in lipoids, which are assumed to be present in cell walls, may account for this. An alternative view is that the urea does not diffuse into the blood stream but is secreted into the blood stream at the proper rate to maintain a constant concentration. Our methods and data do not afford us any means of deciding which of these processes actually occurs but the former seems the more probable.

e. Rather rough estimations of the *crystalloid osmotic tension* of the plasma samples, using the method of calculation mentioned above, show that while there may be some attempt on the part of the organism to keep this tension constant, the compensatory mechanism, if such exists, is not adequate to keep pace with the factors tending to vary the osmotic tension.

3. Urine. When morphine had been given sugar always appeared in the urine in rather high percentage, 5 to 6 per cent. In two cases, dogs 6 and 8, a marked diuresis resulted; in two others, dogs 5 and 7, urinary secretion was practically normal in volume. Dog 9, which had no morphine, showed only a trace of sugar and his volume excretion was normal or slightly increased. As to the fate of the injected glucose, it is evident that it does not stay in the blood and that only a small fraction is excreted in the urine, in fact only a trace when morphine is omitted. It will be noted that the injection is so timed that the anesthetized animals receive glucose at the rate of 0.9 gm. per kilo per hour, the unanesthetized dog's tolerance rate (11). No study of the tissue fluids or glycogen depots was made but we may conclude with Brasol that the remainder is "perhaps changed to glycogen, lactic acid or undergoes some other chemical metamorphosis." The observation of Fisher and Wishart (9) that the metabolism is increased 20 per cent accounts for a part of it.

It is evident, confirming the finding of Meek and Gasser (22), that the gum acacia injected with the glucose does not diminish urinary secretion, as is maintained by Kruse (30); in fact in the cases where hyperglycemia was extreme, with blood sugar values around 0.500 gm. per 100 cc., glycosuria occurred with an accompanying diuresis. Two cases with hyperglycemia and glycosuria, dogs 5 and 7, while not exhibiting a diuresis, certainly did not show a suppression of urine. The urine was not followed in the first four cases. In the case of dog 9, where morphine was omitted with the result that hyperglycemia was not so marked and no significant glycosuria occurred, excretion rate of water was normal or slightly increased. Knowlton (31) finds that colloids exerting osmotic pressure, such as gum acacia, inhibit NaCl diuresis but have but little effect on Na_2SO_4 diuresis. Glucose diuresis belongs to the same class as that of NaCl, i.e., it is presumably due to purely mechanical factors, such as hydremic plethora, rather than to a direct action on the kidney cells. We might explain, then, the normal excretion of urine following the injection of the gum-glucose solution, in the absence of morphine, as due to a balance arrived at between the inhibitory action of the acacia and the diuretic action of the glucose, the later gaining the upper hand when, due to morphine, a marked hyperglycemia is produced with a resultant glycosuria.

SERIES II. ASPHYXIATED DOGS

There is evidence that asphyxia increases the permeability of the vessel walls. Bolton (32) holds that permeability is increased by stagnation of blood with decreased O_2 supply, since edema of the neck is produced by ligature of the superior vena cava, despite the absence of any rise in pressure in the jugulars and presumably in the capillaries. Starling (33) also draws the conclusion that the edema under these conditions must be due to increased permeability of walls which allows protein to pass out into the tissues and hold water there.

The present experiments on asphyxiated animals were carried out with these statements in mind. Study of the data (table 2) shows that, owing probably to the asphyxia, the blood sugar figures are very high, 0.133 and 0.244 before and 0.800 and 0.770 immediately after the injection. In both cases the value 2 hours after the injection was below the initial value, the asphyxia having long since worn off. Except for this no significant departure from the behavior in normal animals occurs. The asphyxia introduces so many complicating circulatory factors, however, that we are not justified in concluding that the permeability of the vessel walls is unchanged, although we have no proof that it is. For example, protein might have been drawn in but promptly squeezed out again due to the asphyxial rise in blood pressure. It will be noted that even without morphine here the hyperglycemia is extreme and the glycosuria marked but that the blood sugar rapidly falls to or below the normal. The apparent continuance of the glycosuria after the blood sugar has fallen to normal is probably due to the fact that the bladder was not completely emptied at each catheterization, urine containing sugar which had really been excreted during the period of hyperglycemia being obtained at each sample.

TABLE 2

Data of series 2, asphyxiated dogs

DOG	SAMPLE	TIME	BLOOD VOLUME	PLASMA CHLOR.	SUGAR	N. P. N.	PROTEIN N				URINE		
							Remaining	Withdrawn	Total	Per 100 cc. plasma	Amount	Sugar	Sugar
		hours	*per cent*	*grams per 100 cc.*	*grams per 100 cc.*	*mgms. per 100 cc.*	*grams*	*grams*	*grams*	*grams*	*cc.*	*per cent*	*grams*
10	1		100	0.635	0.133	25	5.25		5.25	0.980	20	0	0
	2	0	111	0.585	0.800	25	5.55	0.08	5.63	0.881	14	6.4	0.9
	3	2	108	0.625	0.118	26	5.57	0.16	5.73	0.920	40	5.8	2.3
	4	4 35′	105	0.620	0.111	25	5.27	0.24	5.51	0.914	6	5.2	0.3
	5	8	102	0.630	0.182	25	5.15	0.32	5.47	0.930	75	3.4	2.6
Total after injection											135		6.1
11	1		100	0.650	0.244	28	4.77		4.77	0.902	160	0	0
	2	0	116	0.592	0.770	29	4.95	0.07	5.02	0.742	18	+	
	3	2 5′	114	0.640	0.171	28	4.94	0.14	5.08	0.761	12	+	
	4	4 25′	110	0.636	0.167	29	4.74	0.21	4.95	0.770	20	+	
	5	6 45′	102	0.642	0.164	28	4.76	0.28	5.04	0.870	135	+	
Total after injection											185		10.1

TABLE 3

Data of series 3, shocked dogs

DOG	SAMPLE	TIME	BLOOD VOLUME	PLASMA CHLOR.	SUGAR	TOTAL N				URINE		
						Remaining	Withdrawn	Total	Per 100 cc. plasma	Amount	Sugar	Sugar
		hours	*per cent*	*grams per 100 cc.*	*grams per 100 cc.*	*grams*	*grams*	*grams*	*grams*	*cc.*	*per cent*	*grams*
12	1		100	0.632		8.30		8.30	1.004			
	2		88	0.642		6.67	0.08	6.75	0.993			
	3		127	0.592		7.54	0.16	7.70	0.770			
13	1		100	0.629	0.154	10.15		10.15	0.976			
	2		84	0.633	0.145	7.64	0.08	7.72	0.980	24	0	0
	3		129	0.550	0.404	8.32	0.16	8.48	0.707	18	0	0
	4	1 52′	124	0.580	0.154	9.16	0.24	9.40	0.826	26	0	0
Total..........										68		
15	1		100	0.635	0.200	5.74		5.74	0.818	140	0	0
	2		84	0.645	0.220	4.16	0.06	4.22	0.791			
	3		117	0.594	0.444	4.45	0.12	4.57	0.651	10	3.0	0.3
	4	2 10′	114	0.610	0.274	4.40	0.18	4.58	0.672	34	4.2	1.4
	5	4 35′	111	0.640	0.172	4.30	0.24	4.54	0.686	2	4.0	0.08
	6	6 45″	107	0.644	0.141	4.27	0.30	4.57	0.723	0		0
Total after injection..........										46		1.8

SERIES III. SHOCKED DOGS

Dog 12. Body weight, 14.36 kgm. No morphine. Specimens from pancreas, liver, spleen, large and small intestine and kidney for histological study. B. P. at 9:55 is 160 mm. First sample at 10:10, B. P. then 150. Cava clamped at 10:25, B. P. immediately fell to 40 mm. 11:30, B. P. 40, pulse 120, very little ether required. 12:40, B. P. 40, clamp removed, B. P. rose slowly, reached 50 in 3 minutes, 55 in 6 minutes. Second sample, at 12:52, B. P. fell sharply to 38; injection started at 12:57. B. P. soon fell to 34, respirations of expiratory type, heart very irregular. As injection proceeded respiration and heart action improved, B. P. climbed to 40 in 15 minutes. 1:25, B. P. 60. 1:30, animal tried to vomit, intestines extruded from wound, B. P. fell to 40, rose again to 54 in 5 minutes. 1:50, B. P. 60. 1:55, B. P. 65. 1:57, injection ended. 2:03, 3rd sample. Animal tried to vomit during taking of sample 3, B. P. fell to 42. 2:10, B. P. 34. 2:13, animal died. It will be noted that in this severe grade of shock the loss of 13 cc. of blood almost killed the dog (sample 2) and an additional loss of 13 cc. did produce death (sample 3).

Dog 13. Body weight, 18.05 kgm. 1:00 p.m., B. P. 125. 1:20, B. P. 128, 1st sample drawn. 1:25, cava clamped, B. P. fell to 40 and was maintained there. 1:30, ether discontinued. 3:30, clamp removed, B. P. rose to 60 in 2 minutes, amplitude of fluctuations very great. In 4 minutes B. P. averaged 80. 4:00, 2nd sample, B. P. 100, not affected by drawing 13 cc. blood. Injection started at 4:08, 4:20, B. P. 110. 4:30, B. P. 115, ether required. 4:40, B. P. 120. 4:43, B. P. 125. 5:03, 130. 5:08, injection ended. 5:12, 3rd sample, 5:15, B. P. 110, ether almost continuously since 4:30. 5:50, B. P. 90. 6:00, B. P. 80. 6:10, B. P. 64, pulse regular, ether discontinued. 6:25, B. P. 58. 6:40, B. P. 50. 6:55, B. P. 40. 6:57, B. P. 30. 6:59, 4th sample, animal died immediately. This case illustrates well the type of shock in which the blood pressure is well maintained for some time, masking the true severity of the shock; eventually comes the rather sudden terminal circulatory and respiratory collapse.

Dog 15. Body weight, 12.2 kgm. 10:10, 1st sample. 10:20, B. P. 140. 10:25, cava clamped, B. P. fell to 40 and was maintained there. 12:25, clamp removed. B. P. rose to 100 on removing clamp. 12:30, 2nd sample, B. P. 110. 12:35, injection started, B. P. 110. 12:50, B. P. 130. 1:25, B. P. 125. 1:35, injection ended. 1:37, 3rd sample, B. P. not affected by drawing sample. 2:30, B. P. 110. 3:35, B. P. 100. 3:45, 4th sample, B. P. 90. 6:10, 5th sample, B. P. immediately before sample = 80, immediately after = 65. 6:25, B. P. 60. 6:40, B. P. 55. 7:15, B. P. 45. 8:20, 6th sample, B. P. 35 immediately before, dropped to 30 immediately after, death in few minutes.

It is now believed practically universally that in traumatic shock blood plasma leaves the circulation. Inasmuch as the concentration of the proteins of the plasma remaining in the vessels is not appreciably changed it must be assumed that in shock the permeability of the vessel walls is increased. The present series of experiments was planned in order to ascertain whether the fluids drawn into the blood stream by the hypertonic solution in fatally shocked animals would carry along with them more protein than this procedure brings in when applied to normal animals. Four animals were used, but one died in shock before the injection was completed. The results from the other three are given (table 3). Non-protein nitrogen was not followed in these cases as blood was so valuable and it was felt that this determination could best be omitted. The only respects in which the results of this series differ from those of the normal series are in blood volume and protein content.

1. Blood volume. In accordance with the results of numerous previous observers we find the blood volume greatly diminished in shock. In this series the second or "shocked" sample was taken as the standard for subsequent determinations of hemoglobin and blood volume. As a result of the injection of the gum-glucose solution the blood volume is markedly increased above its shock level, the increase even bringing the blood volume above its initial normal level in all three cases. The volume then gradually falls off until (in the one case which could be followed for several hours, dog 15) at the end of 7 hours after the injection it reaches approximately its initial normal level. This was shortly before the animal died.

2. Plasma protein. The method of calculating the absolute amounts of plasma protein may be illustrated here. The case of dog 12 may be taken.

Body weight 14,360 grams

HEMOGLOBIN*				TOTAL NITROGEN†	
Sample	Reading	Volume per cent uncorrected	Volume per cent corrected	Sample	N per 100 cc. plasma
					grams
1	20.0	100	100	1	1.004
2	17.8	89	88	2	0.993
3	25.6	128	127	3	0.77

* Sample 1 is taken as the standard for reading sample 2, sample 2 as the standard for reading sample 3 and any subsequent samples that may be obtained.

† Since N.P.N. was not followed in shocked animals the figures given here as "total N" are the actual Kjeldahl figures. Practically no change in the relations of the figures is produced by the subtraction of the small constant N.P.N. figure from each total N figure, as was done in the first two series.

Calculation. 0.09 × 14,360 = 1292 cc., blood 1.
0.64 × 1292 = 827 cc., plasma 1.
1.004 × 8.27 = 8.30 gm. N. in plasma 1.
Blood 2 has 88 per cent volume of blood 1, i.e. 12 per cent less.

Assume this has all been taken from the plasma. This probably is not correct because of accumulation of corpuscles in the periphery. This peripheral aggregation of corpuscles, however, would, as is mentioned below, make the calculated exchange of protein even greater if it could be quantitatively considered.

12 per cent of 1292 = 155. 827 − 155 = 672 cc., plasma 2.

6.72 × 0.993 = 6.67 gm. N in plasma 2.

Volume of blood 2 is 88 per cent of blood 1, i.e., 1137 cc.

Volume of blood 3 is 127 per cent that of blood 2.

The extra 27 per cent of 1137 or 307 cc. has entered the plasma.

Plasma 2 = 672 cc. 672 + 307 = 979 cc., plasma 3.

9.79 × 0.77 = 7.54 gm. N in plasma 3. Summarizing in the form of a table we have:

SAMPLE NUMBER	PLASMA N REMAINING	PLASMA N WITHDRAWN	TOTAL PLASMA N
	grams	*gram*	*grams*
1	8.30		8.30
2	6.67	0.08	6.75
3	7.54	0.16	7.70

We find that the absolute amount of plasma protein is greatly diminished in shock, its concentration remaining practically constant or being very slightly diminished. This is in agreement with the refractometric findings of Gasser, Erlanger and Meek (34). With the increase in blood volume determined by the injection the absolute amount of protein rises markedly above its shock level but does not reach the normal level. The concentration falls considerably but not to the same degree as plasma volume is increased. In other words, the fluid drawn in is not so rich in protein as is plasma but neither is it protein-free. Due to the method of following the blood volume these figures probably do not indicate the real magnitude of the changes in total protein. As is well known, the slowed circulation of shock causes red blood cells to accumulate in the capillary area so that the number in the arterial blood, in which the hemoglobin was followed, is relatively small and the estimated blood volume therefore high. And it is to be presumed that the improved circulation following the injection of the gum-glucose solution will return some of the jammed corpuscles to the circulation, causing the blood volume estimation to be too low.

Following the blood changes after the injection, it is found that protein continues to increase even while water is passing back out, the protein concentration rising faster than the plasma volume falls. What may happen is that when the injection is given the fluid drawn in brings with it protein through the abnormally permeable walls, but in lower concentration than it occurs in plasma. Then, as the blood pressure rises, the circulation improves and lymph flow is reëstablished. The lymph flow from the liver and intestines is probably accelerated to a far greater degree than that from the extremities, for two reasons: *a*, In these animals the circulation of both posterior extremities was practically done away with since both femoral arteries and veins were ligated. *b*, Since the capillaries of the liver and intestines are the most permeable it is probable that most of the protein that disappeared from the plasma in the process of the development of shock passed out into the tissue fluids of the liver and intestines. As the circulation to these organs is now improved the normal lymph flow is reëstablished and this lymph flow sweeps along with it back into the blood stream through the thoracic duct the plasma protein which had accumulated in the tissue spaces of the liver and intestines during the induction of shock. Thus protein is entering the blood stream even while blood volume is falling. Such an interpretation of the results accounts for the rapid initial increase in blood volume with an increase in absolute amount, although a decreased percentage, of plasma protein, for the subsequent falling off in volume and for the concomitant continued increase in the absolute amount of protein. The prospect of technical difficulties in collecting lymph over a long period of time has kept us from making direct observations on this latter point.

In the shocked animals, as in the normal and asphyxiated, the final sample shows a percentage concentration of plasma protein lower than that of the initial sample and at the same time the blood volume at the time of the final sample is greater than initially; or, as in the case of dog 15, who was followed for 8 hours, when the blood volume has in time fallen to slightly below its initial normal level, the percentage concentration of plasma protein has fallen to a considerably greater extent. This must mean that here too gum acacia is taking the place of plasma protein in holding water in the circulation.

Starling (35) says "Absorption by the blood vessels as a result, say of artificial hemorrhage, if determined entirely by the osmotic attraction of the plasma colloids for the extravascular fluids, can only bring about a passage of water and salts into the blood vessels. . . . According to my explanation this (absorbed) fluid should be pure salt solution. That it is more dilute than plasma is clearly shown by experiments but our data do not yet suffice to determine whether the incoming fluid is a weak solution of protein, such as that contained in the tissue spaces, or is a pure salt solution. If it is proved by quantitative results to contain protein, then some other factor, such as back filtration or active absorption by the endothelial cells of the blood vessels, must be involved in addition to the colloid constituents of the circulating blood." The present data point strongly to the conclusion that some protein does pass in directly in the case of shock although it might not be impossible to explain all of our data on the assumption that the protein is carried back into the blood stream by the lymph. The rapidity with which it occurs, however, speaks against this.

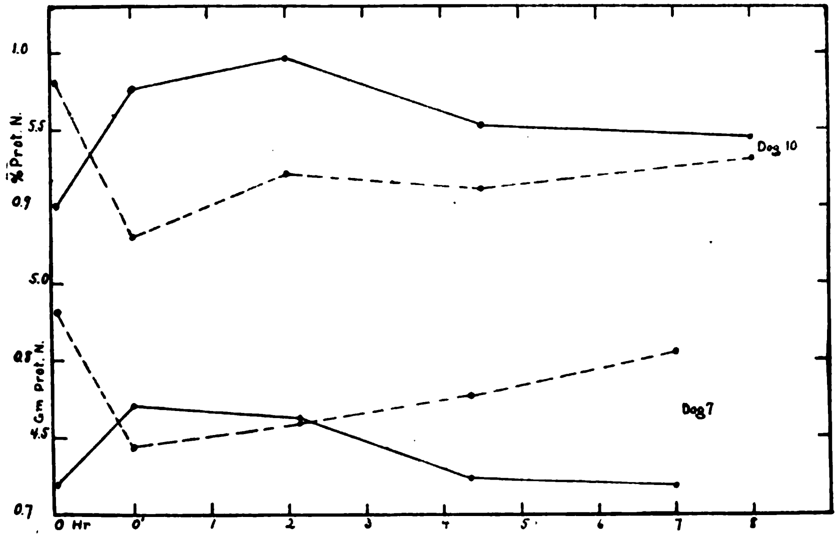

Fig. 1. Changes in total amount (solid line) and per cent (broken line) of plasma protein N in a normal animal, dog 7, and in an asphyxiated animal, dog 10. Time of taking 1st sample is indicated by *0*, the end of the injection by *0'*.

3. Urine. The urine in the case of dog 13, a shocked animal, contains no sugar in spite of the hyperglycemia which far exceeds the normal overflow threshold. This dog was in rather severe shock and the absence of glycosuria is probably due to the fact that no urine was secreted after shock developed. The fact that additional samples of urine could be drawn probably means that the bladder had not been completely emptied of its pre-shock urine. Dog 15 was in better condition and excreted urine containing sugar.

4. Non-toxicity of the acacia-glucose solution. It was our intention in these experiments to produce a grade of shock that would prove fatal in a few hours and in this we succeeded. Some of the animals were almost moribund when the solution was administered. In no case were any untoward effects observed as a result of the injection; the blood pressure, heart action and respiration always were benefited. The rectal temperature was practically unchanged. In one case a slight albuminuria was observed in one sample, but microscopic exami-

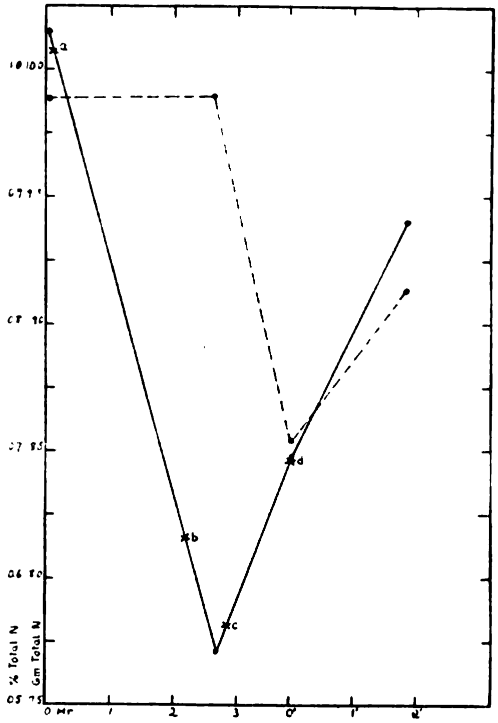

Fig. 2. Changes in total amount (solid line) and per cent (broken line) of plasma total N in a shocked animal, dog 13. Letters designate times of procedures, as follows: *a*—cava clamped; *b*—clamp removed; *c*—injection started; *d*—injection ended. Time of taking 1st sample is designated by *0*, while *1*, *2*, etc., designate hours after taking 1st sample up until end of injection. The end of the injection, when the 3rd sample is taken, is designated by *0'*, while *1'*, *2'*, etc., designate hours after end of injection.

nation revealed large numbers of spermatozoa. The albuminuria had disappeared in the next sample. Casts, hematuria or hemaglobinuria were never observed. No hemolysis occurred as a result of the injections, some slight laking being present in several plasma samples but it was no more evident in samples taken after the injection than in those taken before. This laking is attributed to the ether. These findings in regard to the non-toxicity of gum acacia are in accord with those of Bayliss (36), who discusses the rather misleading statements of Kruse (30) as to the toxic effects observed after the injection of acacia.

SUMMARY AND CONCLUSIONS

A strongly hypertonic glucose and gum acacia solution was injected intravenously into normal, asphyxiated and shocked dogs, and the resultant changes in blood volume and composition were studied.

The immediate effect was a marked increase in blood volume; in normal and asphyxiated animals the blood volume then gradually fell toward but did not completely return to normal in several hours.

The blood volume, markedly diminished in shock, is increased to above its normal level by the injection and then gradually falls to or below its normal level.

The absolute plasma protein is increased slightly or not at all in normal animals and in asphyxiated animals; in an animal which had been bled there was a slight increase when the amount withdrawn was allowed for. The absolute amount of plasma protein is markedly diminished in shock, is increased by the injection and the increase continues for some time after the injection. It is believed that at least a part of the increase in plasma protein following the injection in shock is due to a passage of protein in through the vessel walls.

Gum acacia seems to take the place of plasma protein in holding water in the circulation.

There is a marked hyperglycemia immediately after the injection in normal animals; this is accentuated by morphine and asphyxia. The blood sugar value falls to or nearly to normal within 2 hours. In shocked animals the blood sugar behaves much as in normal animals. There is only a trace of sugar excreted by normal animals excepting when morphine or asphyxia cause marked glycosuria. Shocked animals without morphine excrete some sugar unless, as a result of the shock, there is a suppression of urine.

The fluid drawn into the blood stream brings with it chlorides in concentration equal to the chloride concentration of plasma but the diffusion into the blood stream of sufficient additional chlorides to bring the chloride concentration of injected fluid up to that of plasma is not complete for several hours.

The entrance of urea into the plasma takes place with such facility that the non-protein nitrogen concentration of the plasma remains constant.

There is no suppression of urine in normal animals as a result of the injection, if anything the rate of secretion is slightly increased.

The crystalloid osmotic tension of the plasma does not remain constant.

No hemolysis, hematuria, hemoglobinuria, albuminuria, cylindruria, fluctuations in body temperature or any other untoward effects were observed as a result of the injections.

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THE FUNCTIONAL ACTIVITY OF THE CAPILLARIES AND VENULES

D. R. HOOKER

From the Physiological Laboratory of the Johns Hopkins University

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INTRODUCTION

The significance of the blood stream in the capillary bed for the nutritive processes of the body is well recognized. But it is only in recent times that emphasis has been laid on the capillaries as a factor in the dynamics of the circulation. It is the purpose of this paper to present evidence in support of the belief that the capillaries and venules, as well as the arterioles, respond to direct chemical stimulation and also to indirect stimulation through the intervention of nerve fibers. If this proof is established, the functional activity of the capillary and venous beds must henceforth increase in both theoretical and practical importance. In the first place local tissue needs may by direct chemical action control the capillary blood streaming through the part as shown so clearly by Krogh (1) for muscle. In the second place, the capillaries and venous fields being under the influence of the central nervous system, it follows that local vascular reflexes (2) and systemic vascular reflexes undoubtedly depend upon the coöperation of the capillary and venule with the arteriole; that, in other words, the peripheral resistance, both functional and static, includes arteriole, capillary and venule. And in the third place the effective blood volume, both fluid and corpuscular, must be subject to alteration and regulation to a very significant degree.

Most of the work on the function of the capillaries hitherto reported, except the recent paper by Krogh just mentioned, has been done on the frog in which the transparency of the tissues permits microscopic visualization of these vessels. It is possible, however, using Lombard's method of a drop of oil on the skin (3) to extend such studies to the mammal. Furthermore, most of the recent evidence in the mammal bears upon dilatation of capillaries. It may be assumed that a vessel which is shown to dilate must also contract, but the direct evidence has hitherto been lacking. Nor is there much evidence that the blood capillary and the venule are under nervous control.

HISTORICAL

The first evidence that capillaries have the power independently to change their caliber was published in 1858. In that year Lister (4), describing the early stages of inflammation, pictured with camera lucida drawings a very great increase in the caliber of the capillaries in inflamed tissue. This observation was followed by the studies of Stricker published some seven years later (5). Stricker observed the capillaries in the nictitating membrane of the frog to dilate and to constrict. The constriction was less evident than the dilatation and appeared to be due to two processes, one a nuclear swelling and the other an actual contraction of the endothelial protoplasm. These results were obtained in tissue removed from the body and therefore deprived of its blood supply, and Stricker himself is not convinced that they are not due to lethal changes in the tissue. This possibility is supported by figure 4 in his paper, which pictures a change in shape of a capillary vessel which must be extremely unusual if compatible with functional activity. In a second paper published the following year Stricker (6) describes further experiments with the nictitating membrane of the frog removed from the body and examined in the aqueous humor under the microscope. Many of these preparations were entirely unsatisfactory but a few of them gave beautiful responses to stimulation by ammonia vapor. When the tissue was exposed to ammonia vapor for three or four seconds and then examined under the microscope, Stricker saw the capillary lumina almost disappear and then dilate wide enough for a corpuscle to pass. This phenomenon occurred about twice in fifteen minutes. Further, Stricker observed a varicosity on a capillary which moved forward, suggesting peristaltic activity on the part of the endothelial tube. When the capillaries thus under observation contracted they became practically invisible but could be readily seen again after the contraction had passed off. Stricker likewise employed electrical stimulation. The procedure which he used is not clear but with it he obtained repeatedly good results on various specimens. When stimulated, the capillaries would contract almost instantaneously and dilatation would follow a moment or more after the cessation of the stimulus. After a few applications of the stimulus no further response could be obtained. Even better results were had in the case of the capillaries of the tail of the living tadpole. These vessels were found to respond to mechanical, chemical and electrical stimulation. Of these, chemical stimulation was apparently by far the most effective. Stricker regarded these changes as due to a turgescence of the capillary endothelium such that, without change of the outside diameter of the vessel, the lumen was altered in size due to a thickening of the wall (7).

Although Cohnheim (8), among others, contested Stricker's view that the capillaries possess inherent contractility in the sense just defined, confirmatory evidence was quickly forthcoming. Stricker's work was followed by that of Golubew (9), who confirmed the observation that capillaries change their size under varying conditions, and advanced the observation that certain spindle elements on the capillary walls contract into spheres, thus occluding the lumina of the vessels and so functioning as a constriction. This notion of the mechanism of capillary function was supported a few years later by Tarchanoff (10). This author used alcohol, ether, ammonia, ferric chloride and acetic acid as well as heat. These procedures caused the so-called spindle elements to swell and so to occlude the lumina, but it was not uncommon to find that the vessels failed to dilate after the stimulus was removed.

In 1878 Severini (11) published a monographic study of the capillaries in which he states, among other things, that the application of oxygen causes the capillaries to contract, while the application of CO_2 causes them to dilate. Severini appears to be of the opinion that these gases act chiefly on the spindle elements described by Golubew. The observations were made upon the frog but also upon the capillaries in the mesentery of the guinea pig. Tarchanoff was unable to confirm these observations of Severini's, nor was Roy, working with von Mehring in 1879, able to confirm them. In 1879 Roy and Brown (12) published their very important paper describing observations on the capillary blood pressure in the frog. These authors were convinced that the capillaries have the power of independent contractility and that their caliber is not, as was believed by many, due to passive changes. They found that there was little difference in the size of the capillaries of the frog's web before and after the leg was amputated. Dilatation of the capillaries was also observed on the application of chloroform, and it was also noted that the capillaries dilated as the result of Goltz's "klopfversuch." These authors were of the opinion that it is the whole capillary and not the spindle elements of Golubew which contract. They observed that local anemia produced by compression of the area under observation results in a subsequent hyperemia which is accompanied by a dilatation of arterioles, capillaries and venules. This latter phenomenon occurred after section of the sciatic nerve when stimulation of the nerve caused a strong contraction of the arterioles. It is interesting that they observed only a contraction of arterioles on nerve stimulation. Since this procedure failed to elicit changes in the capillaries, they conclude that the functional activity noted in the latter vessels must be due to some local mechanism and they favor the conception that it is due to a direct chemical action upon the endothelium rather than upon the functional existence of what they refer to as peripheral vasomotor ganglia. The latter conception accords with what we now speak of as axon reflexes, which Krogh has recently invoked to explain localized dilatation of capillaries following punctate stimulation (13). More recently Biedl (1894) saw the peripheral vessels (arterioles, capillaries and venules) all contract in the frog's mesentery on the application of salt solution at 45°C., and dilate again when the heat was removed (14).

Mayer in 1885 (15) again observed the endothelium of the capillaries to contract under electrical stimulation. The evidence that the lymphatic vessels throughout the body are contractile is very strong. The phenomenon was observed by Mayer and by Elliott Clark (16) and others in the tail of batrachian larvae. Histological evidence makes it perfectly clear that the lymphatic capillaries are supplied with nerve fibers. These fibers have been observed and pictured a number of times (Kytmanof, 17), and Camus and Gley (18) have obtained graphic records of the functional response of the larger lymphatics to electrical stimulation of nerves. Sabin, in a comprehensive article on the origin and development of the lymphatic system (19), has collected these data in a convincing manner. The lymphatic and blood capillaries are essentially similar in histological structure. The evidence that the blood capillaries are innervated is not so well established as is the case with the lymphatics, nevertheless there appears to be little doubt of the fact. Shäffer states that nerve fibers may be found to follow each individual blood capillary in the rabbit's mesentery (20). Anatomical knowledge therefore furnishes adequate grounds for the expectation, supported by experimental work on the frog, that the mammalian blood capillaries will be shown to be contractile and under the control of the nervous system.

In 1903 Steinach and Kahn (21) published an extensive paper on the contractility of the capillaries. Their observations were directed almost wholly to the effect of direct electrical stimulation in the frog. They used the excised nictitating membrane from the frog and observed, contrary to the findings of Stricker and Biedl, an actual collapse of the whole capillary tube. According to these writers the collapse is due to activity, not of the endothelium as such, but of the perivascular cells described by Rouget (22) and Mayer (15) so that the endothelial capillary tube is collapsed by a passive infolding of the tissue. The venules were also observed to contract.

Analogous results were demonstrated in the omental capillaries of kittens and guinea pigs. The technical procedure used for these animals is not described and capillaries of less than 10 μ were not seen to contract. They believe that the vessels which they actually saw contract belong, however, to the category of capillaries.

Finally, in a frog preparation which is described in great detail, they obtained contraction in the capillaries of the nictitating membrane upon stimulation of the sympathetic fibers which leave the cord in the third, fourth and fifth spinal roots. The latency of contraction was four to five seconds, sometimes twenty seconds, as compared with a latency of one to three seconds by direct stimulation. It was not uncommon to observe rhythmic contractility of the capillaries after the stimulus had ceased. Galvanic was more efficacious than faradic stimulation and it appears that an exceedingly powerful stimulus was required—twelve Daniell cells.

In recent times attention has been again focussed on the functional significance of the capillary bed largely as the result of the work of Dale and his collaborators in the study of histamine shock (23). Dale has advanced the hypothesis that histamine is an endothelial poison which paralyzes the capillary wall so that a marked dilatation occurs. With this dilatation there results a pooling of blood in the capillaries sufficient to account for the marked fall in blood pressure found in conditions of shock. As the result of the work of Bayliss (24) and Cannon (25) and of Abel and Kubota (26), it would appear that a histamine-like substance may be produced in the body as the result of tissue injury sufficient, under certain conditions, to account for the primary symptoms of shock. So that today the opinion is quite widely held that histamine or a histamine-like body plays an important part in functional processes of the body. This applies not only to pathological states such as shock, but Abel and Kubota have advanced the conception that such a substance is significant in normal physiological processes regulating the distribution of the blood.

In an important contribution to this subject, Krogh (1) has recently shown that an enormous increase in patent capillaries may be demonstrated in active muscle as compared with resting muscle. This increase may indeed amount to more than 700 per cent. Krogh noted that electrical stimulation and massage open up many new capillaries and that electrical stimulation enlarges the capillaries already patent. He regards the mechanism for the regulation of the capillary capacity as being in the capillaries themselves; that is, as due to some chemical regulation or else as associated with an axon reflex mediated through the sensory nerve fibers. He observed that scratching the tongue of an urethanized frog with a glass needle caused a local hyperemia with dilatation of the capillaries and arterioles (13). A closed capillary thus made to dilate opens from the venous end with the appearance of a reversed corpuscular flow. When the dilatation reaches the arterial end of the capillary, the blood stream suddenly assumes the normal direction. Since cocaine was found to abolish this reaction, Krogh was forced to the assumption that it was an axon reflex. He found that cocaine also abolishes the dilatation caused by the application of iodine but does not affect the dilatation caused by acids. Section of nerves supplying the part under investigation at first did not affect the response to chemical and mechanical stimulation. For some days after the nerve section the tissues were hyperemic and the capillaries responded normally to stimulation. Later on, however, the hyperemia disappeared and the capillaries reacted only with a very localized response. From these and other observations Krogh concludes that the capillaries dilate and contract independently of the general blood pressure, and that the spread of response in the capillaries is due to a local axon reflex, probably along the sensory fibers.

Another line of evidence for the contractility of the capillary is found in the tache originally described by Marey (27) in the human being. If a blunt-pointed instrument is drawn across the skin a white line is left which turns to red in a few seconds. In certain cases the red line may be bordered with white and develop a definite urticarial wheal. Clinically this condition is regarded as indicative of vasomotor insufficiency. Marey explained it as due to a localized contraction and dilatation of the capillaries. Bloch (28) thought there was no evidence that the capillaries actually contracted. According to him the red represented capillary dilatation while the neighboring pallor was due to a drainage of blood into the vessels which dilated because of the mechanical injury.

Ryan (29) has sought to standardize this test for application in various forms of fatigue. Cotton, Slade and Lewis (30) found the reaction well developed in cases of soldiers with irritable heart and adduced new evidence in support of the belief that it is a capillary phenomenon. The red line and neighboring pallor may still be demonstrated after the circulation in the arm has been shut off with a sphygmomanometer cuff. Hence they conclude that the arterioles and venules cannot participate in the production of the red line because there is no excess pressure available to dilate these vessels. If the arm be raised before the pressure cuff is applied the reaction cannot be demonstrated because the skin is depleted of blood. On the other hand, if the arm be lowered before the pressure is applied, the reaction is vivid because there is an excess of blood in the skin.

These investigators incidentally show that epinephrin constricts the capillaries. When epinephrin is injected into the skin the resulting pallor is to be regarded as due to a contraction of the arterioles which shuts off the blood from the distal capillaries. If such an injection be made, however, after the blood flow in the arm has ceased as the result of occlusion by a blood pressure cuff, pallor is still produced. Since constriction of the arterioles would now have no effect in stopping the flow of blood into the capillaries, the pallor which results must be due to constriction of the capillaries themselves. This observation that the local application of epinephrin constricts the capillaries is especially interesting in conjunction with the work of Sollmann (31) who showed that histamine also locally applied develops an urticarial wheal.

Very recently Thaysen (32) has reported quite remarkable oscillations in the red cell count in a case of polycythemia. On one occasion he recorded an increase from 5.3 million to 10.6 million in the course of twelve hours. His investigations and tests showed that these fluctuations were caused by varying contraction and dilatation of the capillaries and precapillaries of the skin. This writer is reported to believe that careful observations would reveal similar results in other cases of polycythemia in which the vasomotor system is so unstable that the condition might be called one of vasomotor or capillary ataxia. These observations are an extreme instance of the well recognized difficulty in obtaining consistent red cell counts in many clinical cases. It would be of very great interest and importance to know if such fluctuations are indeed due to alterations in capillary tone.

METHOD

The technique followed in the experiments which form the basis of this paper was very simple. Cats were used exclusively. After anesthesia was established the animal was placed on a flat holder so that the back of the head and ears rested on the same plane with the vertebral column. The ear to be observed was shaved and thoroughly cleaned and dried. By means of a heavy thread through the upper lip the head was so held that an ear flattened out against the board. This flattening of the surface was further accentuated by sealing the ear to the board with collodion. A microscope giving a magnification of about 70 × was adapted for adjustment over the animal holder and a strong artificial light arranged to give direct illumination of the area at an angle of approximately 45 degrees completed the equipment. Castor oil was flooded over the field at the outset and occasionally during the observations.

With such an arrangement a flat vascular plexus can easily be found preferably in the neighborhood of the tip of the ear in which the corpuscular flow in the finer vessels can be readily followed for an indefinite period. In this region the skin lies loosely attached to the connective tissue which forms the framework of the ear. There is no muscle other than that in the blood vessels which might indirectly influence the vascular area under observation. Animals with little pigment in the skin are preferable but it is far from impossible to obtain good visualization of the capillary network and venules even when a considerable amount of pigment is present. One sees vessels of various size, capillaries with red cells streaming through in single file up to larger vessels in which the corpuscles are packed in a thick column. The picture is essentially like that seen in the transparent tissues of the frog. Vessels exhibiting pulsation are infrequent; probably the arteries and arterioles take a deeper course while the capillaries, venules and veins lie quite superficially, as is indicated to the unaided eye. The capillaries ramify freely particularly about the hair follicles and considerable areas are readily found in which the network forms a horizontal plane and therefore is easily visualized without change of focus. It should be emphasized that with the magnification employed one does not see the capillary wall; it is only by the presence of the red blood cells that the capillaries are recognized. Consequently when the vessels are emptied of blood the field becomes a blank so far as the smaller vessels are concerned.

Attention may be directed to the fact that our present conception that the red blood cells course through the capillaries in single file rests upon observations of the capillary circulation in the frog. In this animal the corpuscles are extremely large as compared with the same cells in the mammal (frog 22 x 16 μ, cat 6 μ, man 8 μ) so that the reason for the prevalent opinion is obvious. In the mammalian capillary circulation, including that of man (33), corpuscles may sometimes be seen moving in this manner but it is much more usual to find the capillaries with lumina sufficient to allow more than one corpuscle to pass at a time. This difference is doubtless due to the higher rate of metabolism in the mammal. There is likewise a much more rapid movement of the corpuscles in the warm than in the cold blooded animals. Therefore to restrict the capillary, in the mammal, to blood vessels in which the corpuscles move slowly and in single file is not strictly consonant with the facts.

Post-mortem behavior of vessels. If in the preparation as above described ether be poured down the tracheotomy tube and the animal be thus killed, the movement of blood at first comes to a sudden stop. Then the corpuscles clump slightly and shortly begin to move forward in the normal direction. This movement, at first noticeable in the capillaries, extends to the venules and there is a slow and gradual progression of blood toward the larger veins. The appearance is as if no blood entered the capillaries from the arterial side and that a milking process, akin to peristalsis, swept the corpuscles onward toward the vein. When the process is complete it may be found that here and there in the capillary net a few clumped corpuscles are locked as if the constriction of the vessel had failed to carry on the last of its contents.

These events occupy varying lengths of time in different animals. They may develop completely in a few minutes or they may last half an hour or more. The completeness with which the vessels empty is also a varying factor. Sometimes, particularly in old and debilitated animals, the blood may not be moved at all; sometimes the field is swept absolutely free of blood but more often a few clumps of corpuscles are left stranded, particularly in the venules. The stagnation of these clumped corpuscles is significant in that they give direct evidence of the contraction of the venules since their diameter is readily appreciated to be less than was that of the same section of the vessel prior to death.

The condition of the vessels as thus described prevails for some time, fifteen minutes or more. Then a remarkable change occurs for the vessels begin to relax and fill. Close observation reveals that relaxation first develops on the venous side and that the blood flows, slowly at first, then quite rapidly, from vein to venule to capillary, and that by the end of approximately an hour the vascular area is filled again and filled full with the indication that capillaries invisible before death are now widely open.

One further change remains to be mentioned: an indefinite time after the relaxation and filling of the capillaries just described, the vascular net is once more emptied of blood. This change is roughly coincident with the onset of skeletal muscle rigor and after it is once developed there is apparently no tendency to a reversal, at least after four days there was in one instance no sign of blood in the smaller vessels.

The foregoing description of the post-mortem appearances of the peripheral vascular bed is substantiated by the photograph reproduced in figure 1. In the technique of photographing these vessels two major difficulties had to be overcome, especially when applied to the living animal. The first was to provide sufficient illumination without heat to cut down the exposure time so as to avoid accidental movements. This was accomplished by the use of an arc light projection lantern without the bellows and projecting lenses. The lantern was tilted at approximately forty-five degrees and so placed that the light rays were concentrated almost to a focus on the part to be photographed at a distance of about 60 cm. from the source of light. The second difficulty was to overcome the movements of the ear due to respiration which, however slight, were sufficient when magnified to spoil the picture in the requisite exposure of thirty seconds. In some animals the respiration was quiet enough not to be a disturbing factor but in the majority of cases a clear picture could not be obtained. In the latter, resort was therefore had to artificial respiration for a couple of minutes before the picture was taken. The apnoea which resulted was adequate to the requirements.

For observation alone the nictitating membrane has some advantages over the ear. In the cat it usually offers a perfectly white ground on which the vessels stand out with exquisite definition. Arterioles, capillaries and venules are readily recognized and less illumination is required. Probably the vessels have less covering tissue over them. Salt solution instead of oil must of course be used to keep the tissue moist. A thread passed through the cartilaginous edge may serve to spread the membrane over the eye ball.

This area of tissue is, however, ill-adapted for photographs. It suffers more than the ear from respiratory movements and the arterial pulsation both in the vessels of the tissue itself and in the underlying eye ball cannot be overcome. In addition to these difficulties activity in the smooth muscle of the membrane itself cannot be controlled with the result that the focal plane cannot be maintained. Finally, and most important for the work in hand this tissue cannot be easily brought below the heart level. It follows that passive drainage of the vessels may therefore result if the circulation stops.

The area photographed was magnified ninety times in most of the pictures taken. A larger magnification than this led to trouble because slight differences of position of the plate, which could not be avoided, resulted in a poor focus. The vessels were focussed on the grownd glass plate of the camera at the beginning of an experiment and as a rule this focus was not changed. A focal error frequently crept in, however, due it is presumed to changes in turgidity of the underlying tissues so that the results so far as the relative size of a vessel is concerned are not wholly trustworthy in the case of capillaries. Conspicuous changes in the size of the larger vessels may be relied upon because such changes can be readily recognized by the eye when using the microscope without the camera. As to the capillaries, their presence or absence in the picture should be the sole criterion. If a capillary has disappeared or if its continuity is broken it is proper to assume that it has constricted because, as has been stated, the vessels under inspection lay below the heart level and only active constriction could empty them of corpuscular elements.

Using the procedure above outlined, photographic records were obtained from eight cats in which no preliminary steps were taken other than etherization and tracheotomy. The animals were kept under ether until a satisfactory control picture was taken and then sufficient ether was poured down the trachea to cause prompt death. Of these eight animals three failed to show a primary vascular constriction shortly after death and were not observed further, and six showed complete or partially emptied vessels. The latter group all showed a subsequent peripheral vascular dilatation followed later by emptying. In addition a number of other animals were observed, but not photographed, with confirmatory results.

Figure 1 is selected to exemplify these results. If shows the four stages of vascular change. Three minutes after death the venules are constricted and the capillaries largely emptied. Forty-five minutes

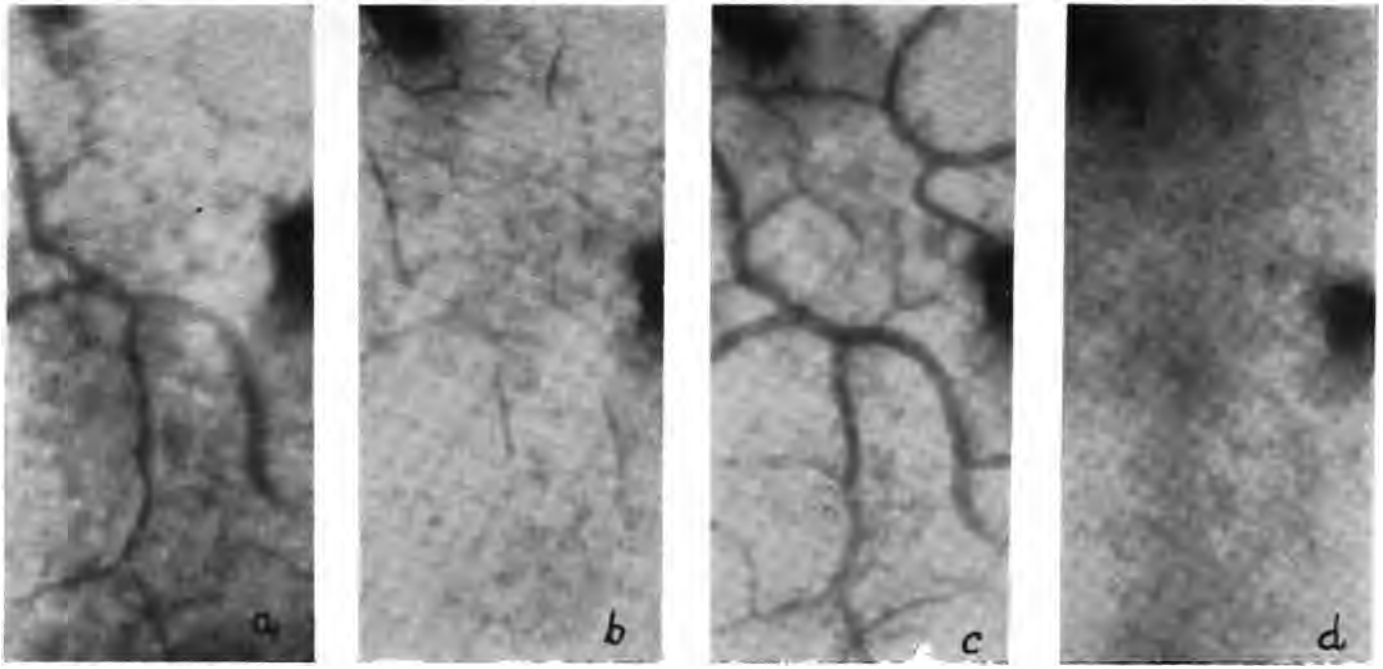

Fig. 1. Post-mortem changes in the peripheral vessels. Magnification 90 ×. Cat. *a*, At 10:28. At 10:36, ether to death. *b*, At 10:39. *c*, At 11:23. *d*, At 12:00. No further change was noted in four subsequent days during which the preparation was preserved. The negatives have not been retouched.

after death the venules are widely dilated and many capillaries are visible. After another period of forty minutes all the blood has been swept out of the vessels and only the shadows of the largest vessels can be found. The data at hand indicate that this last change is permanent since no further alteration was observed to occur for a period of four days.

It may be pointed out here, as was emphasized by Bayliss (20), that there is no inherent difficulty in the conception that the protoplasm constituting the capillary endothelium undergoes change of shape and so mediates a constriction of the vascular lumen. The motility of amoebae and of the white blood cells of higher animals is dependent upon such a change of form and many other similar instances could be mentioned.

That the vascular phenomena above described are not dependent upon the innervation of the vessels was shown in three animals in which death by ether was produced subsequent to section of the cervical sympathetic. In each of these cats the primary constriction was well developed in from one to four minutes. This was followed by dilatation and subsequent constriction in two of the three. In the one which failed to show dilatation the vessels were largely empty three and a half hours after death.

Death by ether subsequent to the intravenous injection of a dose of ergamine phosphate sufficient to cause permanent or transient "shock" (23) is not followed by the same vascular changes. The intact animal or the animal after section of the cervical sympathetic exhibits a primary constriction in the capillaries and venules followed by dilatation and subsequent permanent constriction, as above indicated. If the dose of ergamine is a fatal one or if the animal be killed with ether after partial or complete recovery from "shock," the primary constriction is inconspicuous or wholly absent. In four animals thus observed, two showed no primary constriction whatever and two gave a mere suggestion of it. Furthermore in but one of these animals (a kitten killed by the ergamine) did the constriction subsequent to dilatation develop. In all but this last animal the vessels remained dilated and filled with blood as long as observed (in one case nineteen hours).

These results then clearly support the view advanced by Dale that ergamine phosphate (histamine) is a capillary poison. Additional evidence is found in a single animal in which after histamine and nerve section the vessels similarly failed of the usual response after ether death. In other words, the histamine effect is not dependent upon the integrity of the vascular nerves.

The results thus far presented show that the peripheral vascular bed passes through a number of active changes subsequent to death and that these changes depend upon a local or peripheral function since they are not affected by nerve section and are largely done away with by the injection of an endothelial poison (histamine). Almost at once after death· the capillaries, venules and, presumably, the arterioles constrict with the result that the peripheral field is swept more or less completely free of blood. Since the corpuscles can be seen in transit toward the veins, the result is suggestive of peristaltic constrictions running along the vascular tubes. This condition must be looked upon as the first consequence of asphyxia and may well be a significant factor in the asphyxial rise of arterial and venous blood pressure as ordinarily recorded whereby the lesser and minute vessels throughout the body discharge their contents into the larger channels. This passing constriction gives way shortly to a marked dilatation such as is usually associated with the collection of asphyxial and catabolic products. Some time later and roughly coincident with the onset of skeletal muscle rigor a second constriction develops which is apparently permanent in character. Whether this change is due to a rigor contraction of smooth muscle and endothelium, our present knowledge of these tissues is insufficient to determine. In no case were observations continued long enough (never more than four days) for skeletal muscle rigor to pass off, consequently the assertion that the change, assuming it due to rigor, is permanent is somewhat arbitrary. Post-mortem tissue changes, including laking of the blood, may in this length of time, however, have so clouded the field that the vessels could not be seen even though they were filled with blood. Also the blood may clot and so fail to run into the vessels even after their lumina have become patent. It will be noted that this observation does not accord with the livor mortis seen so frequently by pathologists.

Experiments with nerve stimulation. A second point of even greater interest and importance brought out in this research is the effect of nerve stimulation upon the peripheral vascular bed. In this set of observations the animals were usually anesthetized with urethane and the cervical sympathetic nerve dissected out for stimulation. In no instance was it possible to determine, by inspection or by photographs, that section of this nerve altered in any way the caliber of the vessels of the ear. On the other hand, electrical stimulation of the nerve gave unmistakable evidence of constriction in both capillaries and venules and subsequent to stimulation an over-dilatation was

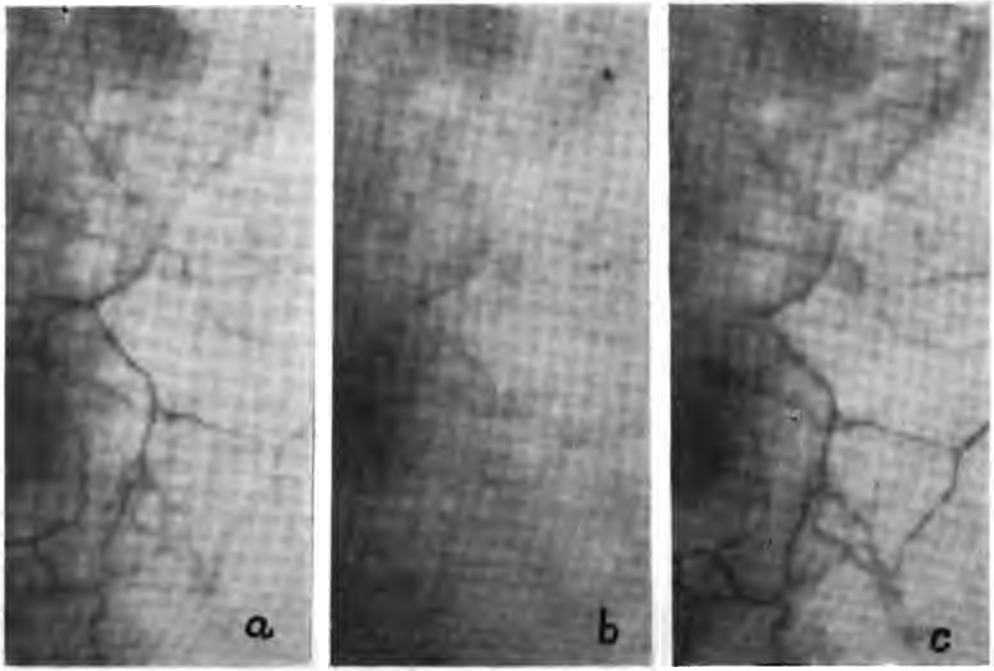

Fig. 2. Constriction of capillaries and venules by electrical stimulation of the cervical sympathetic. Magnification 90 ×. Cat. *a*, Before, *b*, during, and *c*, after stimulation. The negatives have not been retouched.

recognized. These results were so sharp that it was considered necessary to perform but three experiments in each of which the observation was repeated many times. No indication of fatigue was noted since the results were as good five hours after an experiment was begun as at the start. Figure 2 gives the photographs obtained in one of these experiments.

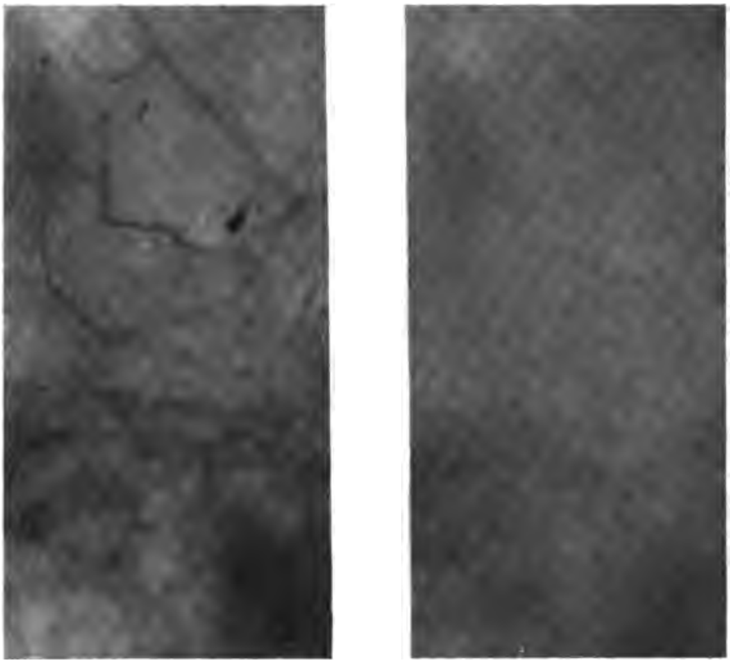

Fig. 3. Constriction of capillaries and venules following the injection of 3 cc. 1: 50,000 epinephrin. Magnification 90 ×. Cat. The cervical sympathetic nerve had been cut. *a*, Before, and *b*, shortly after the injection of epinephrin. The negatives have not been retouched.

This figure shows clearly the constriction and disappearance of the capillaries and venules during electrical stimulation of the cervical sympathetic and their subsequent over-dilatation two minutes after the stimulus was removed. It happens that no larger venule was present in this field but these were repeatedly seen to respond just as conspicuously as shown here for the capillaries. The response of the venules may be appreciated, although not very clearly, in the next figure (fig. 3), which shows the effect of epinephrin injection.

Experiments with epinephrin. Further evidence of the sympathetic innervation of the capillaries and venules was developed from the injection of epinephrin. This substance, selective in action for contractile tissues with sympathetic nerve supply, gave results comparable with those obtained with electrical stimulation of the cervical sympathetic. The effect was the same both before and after section of the sympathetic. Figure 3 is made from photographs taken before and just after the intravenous injection of 3 cc. of 1: 50,000 epinephrin in a cat three and a half hours after the cervical sympathetic had been cut.

The injection of histamine destroys this mechanism. In a cat in which nerve stimulation had given sharp constriction, 6 mgm. ergamine phosphate were injected. As soon as the resultant dilatation of the peripheral vessels was developed, the cervical sympathetic was again stimulated. The strongest stimulating current available failed to elicit the slightest response.

The findings here presented are contrary to our present belief that the active functional peripheral resistance is to be found wholly in the smaller arterioles with smooth muscle in their coats. The evidence given indicates that nerve impulses along vasomotor fibers may play upon the caliber not only of the arterioles but on that of the capillaries and venules as well. We must therefore modify our conception of the peripheral resistance in the matter of functional activity to include the whole peripheral vascular bed including therewith the arterioles, capillaries and venules.

It will be obvious, furthermore, that if the conception in regard to the peripheral resistance here advanced is substantiated, the body possesses a remarkable mechanism for the regulation of the distribution of the blood. For by alterations in the tonic capacity of the capillaries and venules, which is under the control of the central nervous system, circulating corpuscles as well as plasma can be mobilized to a very considerable degree in accordance with the physiological needs of the various tissues both local and general. It would seem also to follow that blood volume and plasma volume determinations must be subject to the same physical mechanism (34).

If, as is highly probable, specific nerve fibers supply the different parts of the peripheral vascular bed (arterioles, capillaries and venules) the play of functional adjustments must be exceedingly complex.. Hitherto it has been possible to invoke chemical processes alone to account for many of the exquisite adaptations recognized to occur in physiological adjustments. In the light of the facts here presented we may conceive of a highly organized nervous mechanism adapted to quick and efficient response superimposed upon the primitive chemical methods available to the organism. There is doubtless a happy coaptation between these two major processes of control but on the body surface, exposed to noxious environmental factors, and in the voluntary muscles where quick adjustments of blood supply are constantly demanded, we might expect the nervous regulation to play a significant teleological rôle. In the glands and deeper body tissues generally, on the other hand, where reaction time is of less significance, responses may largely depend upon chemical factors for their instigation.

If the body has at its control such a highly organized device for the disposition and partition of the blood by which extensive capillary beds may be largely emptied of or packed with corpuscular elements or plasma, an explanation is readily found for the uncertainties of blood cell counts and blood volume determinations. Individual capillaries may be opened up or closed or they may be gorged with stagnant inactive corpuscles, as is possible to demonstrate on the finger (32). Mediation of these and similar changes would be accomplished by activation of the arteriole, capillary or venule functioning individually or collectively. It is natural to infer that normally such forces are well balanced and counteract one another so that the volume and corpuscular composition of the blood is held relatively stable, but in time of physiological stress or in disease the alterations which develop might assume considerable proportions. The significance of this mechanism for the nutrition of the tissues will likewise be apparent since it may be presumed to exercise control over the rate at which plasma passes through the capillaries and into the tissue spaces.

The present work does not include a demonstration of nervous regulation of vasodilatation in the capillaries and venules but the evidence justifies the assumption of such an hypothesis. On the other hand, the primary constriction followed by dilatation which occurs after death indicates that chemical regulation may function both in constriction and dilatation. The effects of the injection of epinephrin and of histamine likewise substantiate this conception. These findings accord with the recent clean-cut results obtained by Krogh (1) on the increase in number of patent capillaries in active muscle and the similar results recognized to occur in the early stages of inflammatory processes (4). The work of Gaskell (35), Bayliss (36) and the writer (37) on the chemical regulation of peripheral resistance is thus to be interpreted that chemical factors constrict as well as dilate the finer vessels other than the arterioles.

To further substantiate the fact that nervous impulses actually produce a constriction of capillaries and venules, attention may again be called to the condition of these experiments. The vascular bed under observation lay some 2 cm. below heart level. An occlusive constriction in the arterioles could not therefore passively drain the vessels distal to the constriction and supporting a hydrostatic column. It is conceivable that, if these vessels were under tension due to a *vis a tergo*, they might decrease in size when their filling pressure was shut off but they would not empty. Indeed it was found in an experiment in which the carotid was occluded long enough to bring the blood stream to a standstill in the peripheral vessels, that the capillaries showed no appreciable decrease in size and that the corpuscles did not tend to clump. The latter point is small but significant because when the capillaries are made to contract, as by nerve stimulation, the corpuscles invariably tend to gather together and move along in clumps.

Lister (4) in that splendid paper to which reference has already been made on the "Early Stages of Inflammation" published in 1858, describes an experiment on the frog which is of decided interest in this connection. He was studying with the microscope the behavior of the peripheral vessels in the web of the foot under various conditions and in this experiment he observed that irritation of the cord caused the capillaries and venules to disappear from view. He ascribes this result to an active constriction of the aterioles sufficient to block the passage of the red cells but insufficient to stop the flow of plasma so that the corpuscles floating in the capillaries and venules are washed onward from the field. The red blood cells of the frog are of course relatively large and could presumably be blocked in the manner indicated, but I am inclined to believe that Lister actually saw a constriction of the vessels in question. In the first series of experiments described in the present paper the evidence is clear that, after the heart has ceased to beat, the capillaries and venules can empty themselves not once but twice against an appreciable hydrostatic resistance. The movement and disposition of the corpuscles under these conditions is not to be distinguished from their behavior under the influence of nerve stimulation; the stream stops quite abruptly, the corpuscles congregate in masses and then progress slowly and without definite regularity. This forward movement occurs against a hydrostatic resistance and in spite of the fact that the corpuscles would tend in a stagnant plasma, because of their specific gravity, to settle and adhere to the vessel wall.

Doctor Connet has recently shown in a research done in this laboratory (38) that the injection of epinephrin raises the systemic venous blood pressure. In this work she was able to exclude the slowing of the heart which has hitherto been regarded as sufficient to account for the phenomenon, and reached the conclusion that the substance acts by a direct effect upon the veins in the intact animal just as it has been repeatedly shown to act upon isolated vein preparations. It seems highly probable in view of the results presented here that the rise in venous pressure following the injection of epinephrin demonstrated by Doctor Connet is associated with a constriction of the capillaries and venules as well as of the veins.

Experiments with histamine. With a method available for the study of the capillaries in the mammal it was quite natural that one should be led to a study of the effect of histamine. The attractive hypothesis advanced by Dale (23) that histamine "shock" (and probably traumatic "shock") is due to a specific toxic action upon the capillaries rested upon indirect evidence. It was possible with the preparation at hand to put this hypothesis to a direct test.

References have already been made to the toxic action of histamine on the capillaries and venules in the experiments previously described:—after the injection of histamine post-mortem constriction of these vessels was absent or very slight and no constriction could be obtained by nerve stimulation. In addition to these experiments a number of experiments were performed, seven in all, in which attention was directed primarily to the histamine effect. Ergamine phosphate, 6 mgm. per kilo in salt solution, was injected intravenously in accordance with Dale's technique.

The results were uniformly clean-cut and decisive. Within a few minutes after the injection the capillaries and venules were filled with stagnant blood and definitely dilated. The dilatation was distinctly more conspicuous in the venules. These changes developed in conjunction with the fall in arterial blood pressure and in one experiment in which it was followed with a fall in venous pressure.

Doctor Rich has obtained similar results in the capillaries of the mammalian omentum (39) by a different method. Rich found that flooding the peritoneal cavity with Zenker's fluid gave prompt fixation of the tissue so that it could be removed and studied under the microscope. If the tissue was thus fixed immediately after the intravenous infusion of histamine, marked capillary and venous dilatation and engorgement could be demonstrated. This vascular change was entirely absent in

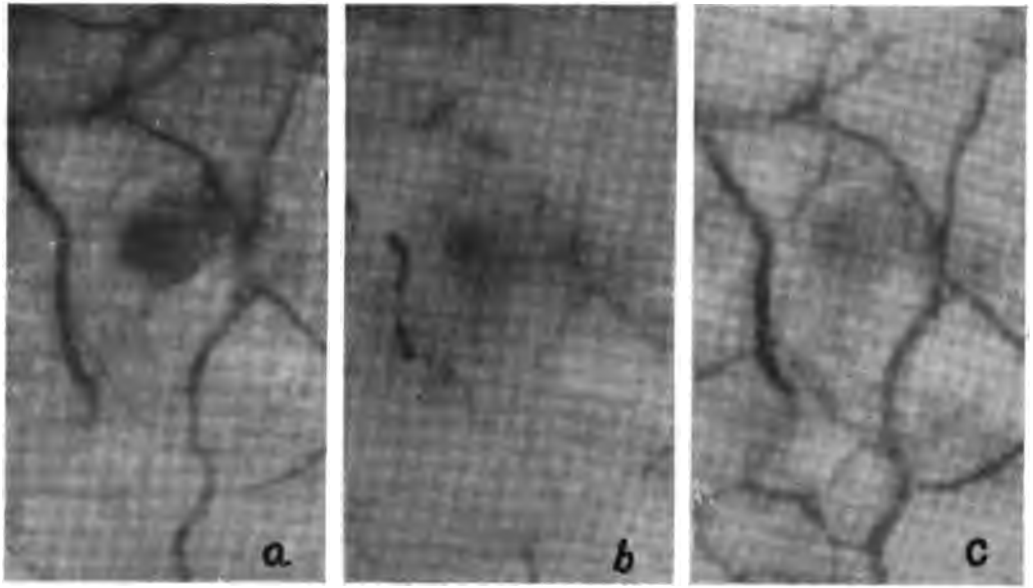

Fig. 4. Effect of histamine on the capillaries and venules. Magnification 90 ×. Cat. *a*, At 10:28. At 10:40, ergamine phosphate (6 mgm. per kilo) was injected. *b*, At 10:50. *c*, At 11:25. The negatives have not been retouched.

control experiments in which salt solution was infused instead of histamine.

Usually, although not invariably in my experiments, this dilatation was preceded by what must be interpreted to be a constriction, under the experimental conditions. This constriction lasted a variable but brief period of time, frequently so short that it could not be photographed satisfactorily. This reaction is especially well exemplified in figure 4 in which the photograph which shows the constriction was taken ten minutes after the injection of ergamine when the arterial pressure was 24 mm. Hg. The arterial pressure was still at 24 mm. Hg. thirty-five minutes later when the last photograph shown in this figure was obtained.

This transitory constriction of the capillaries and venules may not occur throughout the body since Rich was unable to find any evidence of it in the capillaries of the omentum. It may be due to a primary central effect of the poison or, what seems more probable at the moment, it may represent one of those curious reactions according to which a drug or substance depressive in effect at first acts as a stimulus. Such a transitory reversal of effect is not uncommon in perfusing the isolated heart with inorganic salts and I have observed a similar effect in perfusing the respiratory center (40). Burridge (41) has suggested, in the case of the heart, that the condition is associated with the state of aggregation of the colloids.

Although it thus appears possible that histamine may under certain conditions produce its primary "shock" effect while the capillaries and venules of the ear are constricted, these experiments as a whole undoubtedly lend strong support to Dale's hypothesis to explain histamine "shock."

In conclusion it will not be out of place to state that the preparation used in these experiments offers an excellent method of demonstrating the capillary circulation in the mammal to students. A low power microscope (ocular 1 and objective 3), adapted by removal of the stage to fit over the edge of an animal holder and a good light are all the apparatus that is required. The capillary circulation can be similarly observed in the rabbit and presumably also in the dog although the latter animal has not been investigated. The rabbit's ear is, however, distinctly more susceptible to inflammatory processes than is that of the cat.

SUMMARY AND CONCLUSIONS

A method is described whereby the peripheral circulation (particularly the capillaries and venules) in the cat's ear may be observed and photographed. It is thus possible to study in the living mammal the capillary circulation, investigation of which in the intact animal has hitherto been limited to the frog. Making use of this method, the following experimental results were obtained:

1. After ether death the peripheral vessels at first constrict so that they are largely emptied of blood. This constriction which occurs usually within a few minutes lasts but a short time and is followed by a marked dilatation and engorgement. Subsequently and roughly coincident with the development of skeletal muscle rigor, the vessels are again emptied of blood. The latter condition prevails indefinitely (four days at room temperature).

These changes are much less conspicuous or entirely absent if the animal is previously injected with histamine. They are not affected by section of the vasomotor nerve fibers (cervical sympathetic).

It is thus concluded that these post-mortem vascular reactions are independent of the central nervous system and that they are abolished by an endothelial poison.

It is suggested that the first constriction represents a response to asphyxia and may be a significant factor in the asphyxial rise of arterial and venous blood pressure.

2. Section of the vasomotor fibers to the part (cervical sympathetic) did not cause an appreciable dilatation of the vessels but electrical stimulation of these fibers gave clear evidence, by causing constriction, that the capillaries and venules are under sympathetic nervous control. This fact was further substantiated by the injection of epinephrin which caused a similar vascular response.

The reaction to nerve stimulation could not be obtained in an animal poisoned with histamine.

These results indicate that the functional peripheral resistance is not limited to the arterioles but includes the capillaries and venules as well. It is inferred that this resistance is subject to chemical as well as nervous control. This concept involves a reorganization of our present beliefs concerning the peripheral resistance and implies the existence of an efficient physical mechanism for the distribution and regulation of the circulating blood volume and of the supply of nutriment to the tissues.

3. The injection of histamine (ergamine phosphate) causes a prompt and permanent dilatation of both capillaries and venules with stagnation of the corpuscular stream.

This reaction appears to be characteristic and thus confirms Dale's hypothesis that histamine "shock" (and presumably traumatic "shock") is largely dependent upon the reaction of the capillaries. It should be noted, however, that the present results extend this reaction to include the venules as well as the capillaries.

Prior to the dilatation which appears to be the characteristic histamine effect, there is usually a short period when the vessels in the ear are constricted. Neither this transitory effect nor the general histamine effect is influenced by nerve section. Both effects are thus due to the direct action of the substance on the vessels.

It is probable that vessels elsewhere in the body do not show this passing constriction since it may persist for some minutes after the systemic arterial pressure is at shock level and give place to dilatation while the blood pressure level remains unchanged.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

I. Lung Automatism and Lung Reflexes in the Frog (R. pipiens and R. catesbiana)

A. J. CARLSON and A. B. LUCKHARDT

From the Hull Physiological Laboratory, University of Chicago

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This report is the beginning of an investigation and analysis of the reflexes evoked by the visceral sensory nerves in all the groups of vertebrates available for study. The inception to this line of work was the observation on man (7) that strong contractions of the empty stomach produce reflex effects on the cardiac and the vasomotor centers. To date we have studied the reflexes from the visceral afferent system involving the skeletal musculature, the respiratory mechanism, the gastro-intestinal tract, the heart and blood vessels, and the urinary bladder. In some cases our reflex results compelled us to re-investigate the motor mechanisms of the organ involved in the reflex response. This is true especially of the lungs.

We have today fairly comprehensive and accurate knowledge of the efferent nervous mechanism of the viscera, thanks to the work of Gaskell, Langley and others.

On the sensory or afferent side our information is made up largely of gaps and guesses, despite its probable importance in functional integrations in health and disease. This phase of physiology has been studied especially with reference to *conscious* visceral sensations, to witness only the work of surgeons and internists on direct and referred visceral pain, and of physiologists and psychologists on the sensibility (conscious) of the alimentary canal. To our knowledge a thoroughgoing investigation of the sub-conscious reflexes evoked from the visceral sensory nerves in health and disease has not been made. In Gaskell's recent monograph (8) on the involuntary nervous system the afferent component of this system is not even mentioned, and in Sherrington's article on the "Sympathetic Nervous System" in the 1911 edition of the Enclyclopedia Britannica the afferent component is dismissed with the following sentence: "Of the afferent fibers of the sympathetic little is known save that they are, relatively to the efferent, few in number, and that they, like the afferents of the cerebro-spinal system, are axones of nerve cells seated in the spinal ganglia."

EXPERIMENTAL METHODS

1. The lung contractions were registered by means of water manometers (diameter 8–10 mm.) connected with small glass cannulae inserted and tied in the tips of the lungs. For the most delicate lung contractions these water manometers were not sufficiently sensitive, and in the study of these phases a very delicate tambour was employed. In fixing the cannula in the tip of the lung care must be taken so that the direct handling of the lung is minimum and gentle, as direct and rough handling induces prolonged tonic contractions that may involve the whole lung. In animals in poor physiological conditions, the lungs are usually quite atonic, and these contractions due to direct handling (mechanical stimulation) are less in evidence.

In experiments with the glottis open and the frog preparation breathing spontaneously no artificial pressure can be maintained in the lungs, because if the lungs are collapsed through cannulae in the lung tips, the frog promptly fills the lung again up to the original pressure. If this original pressure is slightly exceeded by inflation through the cannula the glottis is promptly opened and the pressure reduced. In the experiments involving the closure of the glottis the lungs were practically always collapsed and empty at the conclusion of this operation In the subsequent inflation of the lungs we always took pains not to exceed the normal pressure maintained by the frog (1–3 cm. water).

Most of the previous investigators of the physiology of the respiratory movements in the frog have used various methods for graphic registration of the throat and flank movements (Martin (16), Wedenskii (26), Langendorff (14), Sherrington (23), Baglioni (3), Soprana (24), Nikolides (20), (21)). Brown (5) and Willem (27) recorded intrapulmonic pressure by means of cannulae in the tip of the lungs. Mochi (17), (18), (19) placed the body of the frog, except head and throat, in a plethysmograph and closed the plethysmograph by sectioning the frog's skin around the neck and tying to the plethysmograph tube. It seems to us that the method of Mochi introduces more trauma and abnormal physiological conditions than a slit through the abdominal wall for placing cannula in the tip of the lungs.

2. For the registration of the variations in the intrapulmonic pressure during normal respiration the animals were usually decerebrated, and slits made through the abdominal wall over the lung tips, of sufficient size to insert cannulae in the lung tips. This incision through the abdominal wall was made with or without local application of cocaine. The animals were then placed, usually without restraint, on a board or preferably in a small dark box. In most cases animals thus prepared would sit quietly for long periods, unless disturbed by external stimulations. In a few animals the abdominal incisions were made under local anesthesia without previous decerebration.

3. In the experiments where it was necessary to separate the lungs completely from the influence of skeletal muscle contractions several methods of procedure were used:

a. After decerebration and fixing the cannulae in the lung tips, the animals being placed in normal position (ventral side down) on the board or in the dark box, the abdominal muscles were cut away and the spinal cord pithed below the brachial plexus. In such a preparation movements of the head, strong respiratory movements (swallowing) or movements of the front legs will alter the intrapulmonic pressure, but the rapidity of these movements is much greater usually than the lung contractions so that the latter can be readily differentiated from the passive effects of the former movements, and in favorable preparations the former movements may be absent over considerable periods, thus giving the lung contractions free play.

b. Without previous decerebration the spinal cord was cut just below the medulla and pithed the entire length caudad, the animal placed on the board dorsal side down, the abdominal wall opened for its entire length by a median incision, and the lungs completely isolated, except for their anatomical connections with the pharynx and esophagus. Animals thus prepared continue to breathe spontaneously for considerable periods if care is taken not to injure the lungs or pharynx and prevent exsanguination. Head and pharyngeal movements are still capable of influencing the intrapulmonic pressure mechanically. The isolated lungs were prevented from drying by a thin layer of absorbent cotton kept moist with Ringer's solution.

4. Closure of the glottis. Our greatest technical difficulty consisted in proper closure of the glottis in experiments where this procedure was essential. The frog has no trachea and bronchi. The glottis opens directly into a rather large tracheal sac which communicates with the base of each lung. This tracheal sac is so closely adherent to the tissues at the base of the heart that we found it impracticable to close the lungs by ligation or compression of this sac without an amount of injury to the heart nerves, and main blood vessels that resulted in quick failure of the circulation. The following experiments were tried without practical success:

(1) Ligation of base of lungs at their junction with the tracheal sac, leaving the lung blood vessels and nerves outside the ligature. This failed because of the impossibility of accomplishing the latter without puncturing the lung wall, or if successful the ligation produced enough anatomical distortion to interfere with the lung circulation.

(2) Closing the tracheal sac or either lung by small wads of cotton pushed through the glottis. This failed mainly because the glottis opening is smaller than the diameter of the tracheal sac or its communication with the lungs. Hence the cotton wad passed sooner or later into the lung cavities.

(3) It was noted, when the median incision exposing and isolating the lungs was carried forward to the level of the base of the heart only, that lateral and dorsal tension exerted by pull on the front legs would prevent air from entering or leaving the lungs by the normal breathing movements. This was evidently due to collapse of the tracheal sac by external compression. This gave us the clew to a method of closing the glottis and blocking the air communication between the two lungs with the least possible trauma or physiological violence. A cotton plug of suitable size, with or without a coating of vaseline, was inserted through the mouth and pushed down the esophagus to the level of the glottis and the tracheal sac. The pressure thus exerted on these structures from the esophagus not only closed the glottis but usually compressed the tracheal sac sufficiently to prevent air communication between the two lungs, especially if in addition the front legs were put under slight dorso-lateral tension.

This mode of procedure sufficed for the degrees of lung contractions accompanying the normal respiratory movements, or induced by reflex stimulation. But it proved inadequate in case of the extreme tetanus of the lungs following section of the vago-sympathetic nerves or pithing of the medulla. These strong contractions always forced the glottis open against the cotton plug in the esophagus. Hence in all the experiments on this phase of lung physiology the glottis had to be closed more firmly. This was done by clamping the rim of the glottis with a slender artery forceps. The mouth being held open, a slender hook was passed through the glottis, under gentle forward traction the rim of the glottis was compressed with a slender artery forceps, a small cotton plug pushed into the esophagus and left in situ together with the forceps. Care must be taken not to place the forceps too far down on the tracheal sac and pharyngeal tissues, as in that case the pulmonary branches of the vagi as well as the lung blood vessels are included in the grip, or placed on such tension that vagus action on the lung and lung circulation are interfered with.

The essential drawback to this procedure is the trauma produced or rather the violent mechanical stimulation of the sensory nerves in the glottis, larynx and pharynx by the compression. Placing the artery forceps in the region described produced something like profound prostration or "shock" in the preparation. Respiratory movements cease for a considerable period, and in the case of animals otherwise in poor condition may not return at all. It is scarcely necessary to add that the results reported, using this method of closure of the glottis, are based on the vigorous preparations in which spontaneous respiration returned.

5. The mucus in the lung cavities can, of course, not be eliminated from the lungs in the normal way under any condition of glottis obstruction. Furthermore, the unavoidable trauma to lungs and tracheal sac in preparation may actually increase the mucous secretion. This lung mucus is a hindrance and a source of error in registering lung tonus and contractions by our method, as strong contractions may force some mucus into the cannula in the lung tips, and this will interfere with the prompt and accurate response of the water manometer to slight variations in the intrapulmonic pressure. It is needless to say that preparations must be discarded in which the lung mucus interferes with accurate recording of lung contractions.

6. *Administration of the drugs.* All the drugs used, unless otherwise noted, were mixed with varying quantities of Ringer's solution and injected slowly into the abdominal vein. A few injections were made directly into the heart.

7. *Prevention of asphyxia after closure of the glottis.* It was, of course, essential to maintain circulation and lung ventilation even after closure of the glottis, so that abnormal reflexes and local lung reactions would not be set up by asphyxia. We endeavored to maintain good circulation by ligation of the main blood vessels sectioned in the preparation of the animal and by occasional intravenous injections of small quantities of Ringer's solution. The frog's heart is apparently very sensitive to the mechanical factors of filling, as it ceases to beat entirely or beats

very feebly when the blood pressure is very low, but resumes an adequate rhythm on replacing the lost blood with Ringer's solution.

It is well known that the frog in water carries out a considerable proportion of its gaseous exchange through the skin. Under the temperature conditions prevailing in the laboratory during this work the frogs (R. pipiens) would remain under water for 18 to 25 minute periods, come to the surface and make a few vigorous respirations and submerge again for the same length of time. Evidently the filling the lungs with air, supplemented with the skin respiration, met the respiratory needs for 20 to 30 minutes. On the basis of these facts, we always kept the skin of our frog preparations moist with water, and gave occasional artificial respirations, except in cases of extreme lung tetanus when the latter procedure would have been useless.

8. All the tracings reproduced with this report were taken with the same speed of the kymograph. The time record is not always attached to the tracings reproduced, for reasons of economy of print paper. But the reader interested in any question involving the time element as a matter of importance can readily transfer the time tracing, given in a few of the tracings, to the others; 25 cm. of tracing (original size) = 17 minutes.

LUNG TONUS AND LUNG CONTRACTIONS DURING NORMAL RESPIRATION

1. The anatomy of the frog's lung is well known. The reader will recall that the lung is a paired muscular sac, numerous septa on the interior surface dividing this into small spaces or alveoli. The septa extend only a few millimeters from the lung wall, so that the larger part of the lung cavity is a large single air space. There are no bronchi and no true trachea, the tracheal sac having essentially the same structure as the rest of the lungs, and probably carries out the same respiratory function.

Smooth musculature covers the entire wall of the lungs and extends into the smallest septa on the inner surface. More or less definite external muscle strands follow the course of the main pulmonary blood vessels on the lung surface.

The arrangement of the lung musculature is such that contraction (even of the septal musculature) will reduce the size of the lung cavity, or raise the intrapulmonic pressure in case the air in the lung is not free to escape.

The action of the septal musculature would be analogous to that of the bronchial constrictor muscle of the mammalian lung. So far as we know, the mammalian lung has no counterpart to the lung wall musculature in the frog.

After having discovered the striking peripheral motor automatism of the frog's lung we become especially interested in the local nervous tissue in the lung of this animal group. According to the histological investigations of Arnold (1), Smirnow (25), Cuccate and Wolff (28), there are numerous ganglia, as well as isolated ganglion cells (multipolar and bipolar) along the course of the main vago-sympathetic nerve trunks on the surface of the lungs. There are medullated and non-medullated nerve fibers in these nerve trunks. A plexus of fine non-medullated nerve fibers surrounds the strands of lung musculature. The ganglion cells and these nerve plexuses are most abundant at the base of the lungs. Arnold points out that the ganglia and ganglion cells in the frog's lung are histologically identical with those of the frog's heart. They are also probably identical, both as to histology and function, with the ganglionic plexuses (Auerbach) in the wall of the gut, especially as the lung is a diverticulum from the esophagus.

2. The external respiratory mechanism of the amphibians differs from that of all other air-breathing animals in that the air enters the lungs under positive pressure due to the act of swallowing. One might therefore surmise that in the amphibia the respiratory center in the brain is anatomically and physiologically identical with the center for deglutition.

The sequence and coördination of the respiratory acts (buccal movements, closing of nares, expiration and inspiration or swallowing air) have been correctly analyzed and described especially by Langendorff (12), (13), Baglioni, Brown (5) and others, and most recently by Willem (27). We have nothing new to add on that point, and can contribute no new facts bearing on the old and new speculations as to phylogenetic and physiological significance of the buccal movements which proceed rhythmically between the actual renewal of air in the lungs (swallowing).

The types of the respiratory rhythm as revealed by the intrapulmonic pressure in normal and in decerebrated frogs are shown in figure 1. The filling of the lungs by swallowing (upstroke) is preceded by opening of the glottis and escape of some air into the buccal cavity. According to most of the competent and recent investigators, the nares are closed during the whole act so that while the air escapes from the lung it does not actually escape through the nares. Rebreathing is therefore a marked feature of the frog's lung respiration. The buccal movements going on between the swallowing acts and with the nares open, bring fresh air into the buccal cavity.

In exceptional cases there is a perfect synchrony between the buccal movements and the actual air swallowing (fig. 1, *B*). But usually several buccal movements are made between each swallowing act (fig. 1, *A*), and a striking feature of the air swallowing rhythm in most frogs is a periodicity similar to the Cheyne-Stokes breathing in mammals. This has been observed by most of the former investigators on the respiratory movements in the frog (Luschinger and Sakalow (15), Langendorff (12), (13), Wedenski 26, Sherrington (23), etc.). The

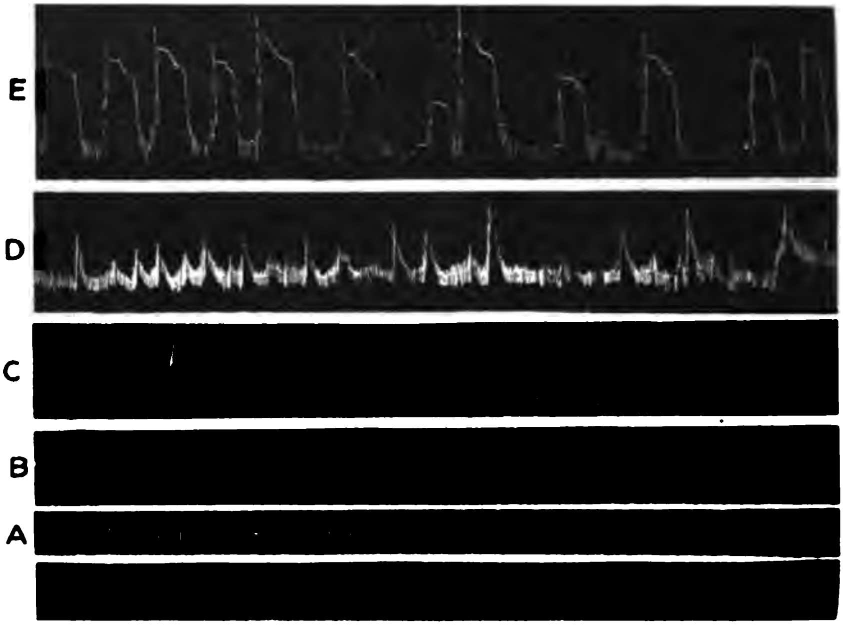

Fig. 1. Records of intrapulmonic pressure in frogs. Tracings *A*, *D*, *E*, taken by water manometer; tracings *B* and *C* by air transmission and tambour. Tracings *A* to *D*, Rana pipiens, animals decerebrated and the tip of one lung exposed by small abdominal incision. No anesthetics, animals sitting quietly in normal posture. Tracing *E* from bull frog (R. catesbiana), tip of lung exposed by small abdominal incision after cocaine, animal sitting quietly in a darkened moist box; time, 5 seconds. Showing varying types of respiration in the frog

All the tracings in this article are reduced to about $\frac{2}{3}$ of the original.

frog may go on over long periods swallowing as much air as that which previously escaped through the open glottis, thus maintaining a constant general level of intrapulmonic pressure of 1 to 2 cm. of water. This type may periodically change into one in which during a few powerful air swallowings the amount of air forced in greatly exceeds the quantity that escaped between the time of glottis opening and the swallowing act. In consequence of this the lungs expand and the intrapulmonic pressure rises from the general level of 1 to 3 cm. of water up to a level of 6 to 9 cm. of water. All respiratory movements then cease for periods varying from 5 to 60 seconds and the act is renewed, that is, the quantity of air let out of the lung in each respiratory act is greater than that forced in; the lung shrinks and the intrapulmonic pressure falls to its former general level of 1 to 2 cm. of water (fig. 1, *C, D, E*).

According to our experience, this is the usual type of respiration in the frog. As previously noted by Sherrington and others, decerebration or other methods of preparation are not responsible for inducing it. It is probably a normal rhythm developed in connection with the habitual under-water existence of the animal. During the respiratory pause with high intrapulmonic pressure this pressure, as recorded by the ordinary water manometer, may show an initial rise and then remain at a fairly constant level until the next respiratory act, but if the pause is long the intrapulmonic pressure gradually falls, due, not to escape of air through the glottis, but to relaxation of the tonus of the lung musculature.

3. Active lung contractions and lung inhibitions associated with the respiratory movements.

a. Contractions. The reader's attention is invited to the tracings reproduced in figures 2 and 3. It will be noted, especially on the upper tracing in figure 2, that at the end of the last respiration followed by a Cheyne-Stokes pause, there is a latent period of 1 or 2 seconds followed by a rise in the intrapulmonic pressure that may exceed the maximum upstroke of the final inspiration. When the pause is sufficiently long and registration apparatus sufficiently delicate, it will be seen that this rise in pressure is due to a contraction lasting from 10 to 15 seconds. These contractions are evidently due to the activity of the lung musculature, for we have observed them in animals after isolation of the lungs, fixation or resection of the abdominal and shoulder muscles or destruction of the entire spinal cord below the medulla. Moreover, the changes in the intrapulmonic pressure due to active

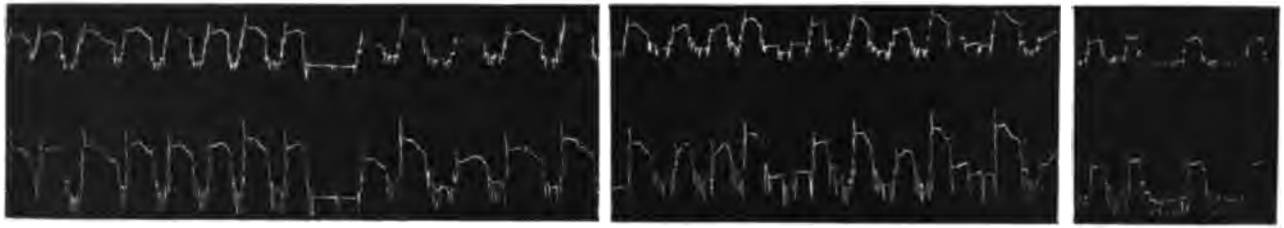

Fig. 2. Tracing of intrapulmonic presure in the bull frog in normal respiration. Frog in normal posture and resting without restraint in dark box. Operation for insertion of cannula in tip of one lung made under local anesthesia. Lower record by water manometer, upper record by delicate tambour. Showing the curve of lung contraction during the respiratory pause following the periodic rapid inspiration. The lung contractions are shown best on the tambour tracings, as this instrument is more delicate than the water manometer.

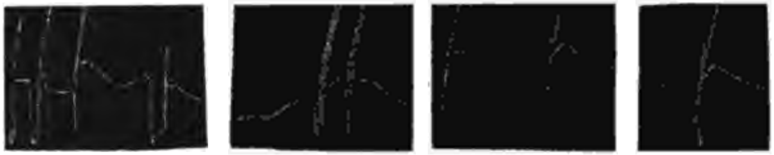

Fig. 3. Rana pipiens. Water manometer tracings showing contractions of lung following spontaneous respiration (quick up and down strokes). Whole brain exposed, spinal cord cut and pitted below medulla. Cannula in tip of one lung, opposite lung tied off. Glottis open. Animal lost much blood and was breathing irregularly.

contractions or relaxation of the skeletal musculature are more rapid. The contractions are also too slow to be due to passive elastic rebound of the connective tissue of the lung. They are, however, very similar to the quick spontaneous contractions that are seen at times in the hypertonic frog lung after cutting the vagi or complete destruction of brain and spinal cord (fig. 7). The tracing in figure 3 illustrates the fact that these lung contractions may follow single respiratory movements, if the pause between two successive swallowings is of sufficient duration.

It would seem that these contractions of the lung musculature following the active inspiration or attempts at inspiration have not been seen by previous workers, except possibly Graham Brown (5). On some of the tracings published by Brown there is an increased pressure in the lungs shortly before expiration, and Brown suggests that this is due to muscular contractions in the lungs.

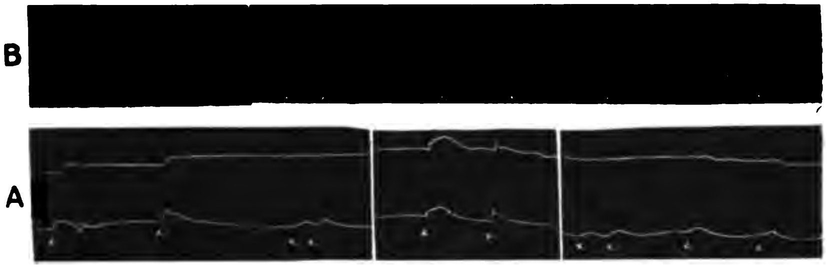

Fig. 4. Water manometer tracings of the contractions of the lung musculature in the frog (Rana pipiens) that follow upon the external respiratory movements. Spinal cord transected and destroyed below the medulla. Lungs isolated and cannulated (tip). Glottis closed with forceps so that there is no communication between the two lungs. *A*, upper tracing equals left lung; lower tracing equals right lung. *B*, same. *X* = pharyngeal respiration or attempt at swallowing air.

The lung contractions do not depend on the change in tension on the lung tissues following a forceful inspiration. The glottis may be closed and the pressure in the lungs raised to that of the normal of 1 to 3 cm. of water, and in such preparations each attempt at respiration, single or a series, is followed by lung contractions (fig. 4). In preparations with the glottis closed the contractions are usually more prolonged than those seen in figures 2 and 3.

We are thus forced to the conclusion that in the frog the normal inspiratory movements lead to active contractions of the lung musculature through associated innervation, either of the motor fibers to the lung or through central depression of the inhibitory fibers controlling a peripheral automatism.

b. Inhibition. In preparations with the glottis closed, and in intrapulmonic pressure approximately normal (1–3 cm. of water), *the active respiratory movements lower the intrapulmonic pressure,* evidently by inhibition of the lung muscle tonus (fig. 5). With the glottis closed no air can enter or leave the lungs. The respiratory movements of the throat and pharynx, especially if they are vigorous, induce slight fluctuations in the intrapulmonic pressure of equal rapidity with the pharyngeal movements. Vigorous movements of the head may induce, possibly, stronger positive pressure in the closed lungs synchronously with these movements. It is possible but not probable that the lowering of lung muscle tonus during the rapid and rigorous swallowing movements are due to mechanical stimulation of the lung from this source, since the inhibition cannot be produced by similar fluctuations in intrapulmonic pressure artificially induced, and direct mechanical stimulation of the lungs when strong enough to have an effect causes contractions.

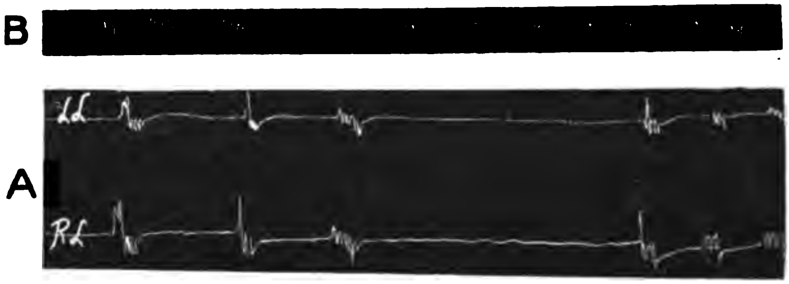

Fig. 5. Water manometer tracings of the intrapulmonic pressure in frogs (R. pipiens). Spinal cord cut and pithed below medulla. Cannula in tip of lung, and glottis closed (imperfectly) by cotton and collodion. *A*, simultaneous record from both lungs of the bull frog. *B*, record from lung of R. pipiens. Showing inhibition of the tonus of the lung musculature during the active respiratory movements. The rapid fluctuations (respiration) on the tracings are due to movements of larynx and head, and not to entrance and exit of air into the lungs.

The return of the lung muscle tonus following the period of inhibition is quite similar to the lung contractions described in previous sections and illustrated in figures 2, 3 and 4. So far as we know, this inhibition of the lung musculature has not been noted by previous investigators.

The utility of the correlation is obvious, the relaxation of the lung musculature during inspiration being favorable to the filling of the lungs by the swallowing act.

THE PERIPHERAL MOTOR AUTOMATISM OF THE LUNGS AND THE INFLUENCE OF THE VAGI AND THE CERVICAL SYMPATHETIC NERVES ON THIS AUTOMATISM

1. In preparations with the glottis closed, lungs isolated from the influence of skeletal muscle contractions, section of the vago-sympathetic nerves in the neck, destruction of the medulla, or ligation of the base of the lungs *induces immediately a permanent hypertonus or incomplete tetanus of the lung neuro-muscular mechanism* (figs. 6, 8 and 9). By permanent, we refer, of course, only to the time of observation in these crucial experiments, that is, 2 hours. We found it difficult to maintain the preparation in good physiological condition over longer periods, especially with both lungs contracted down so that the lumen is completely obliterated thus preventing lung ventilation. The circulation also fails gradually.

The hypertonus of the lungs is usually at its maximum shortly after the isolation from the central nervous system, and there may be a gradual fall of the tonus during the observation period of 1 to 2 hours. This gradual fall is probably due to the failure of maintaining adequate circulation in the lungs, that is, to asphyxia.

This remarkable lung reaction is obtained in all frogs in good physiological condition, and the better the condition of the frog the stronger the lung tetanus on sections of the vagi. In frogs in poor condition (infected, starved or moribund from any cause) destruction of the medulla or vagi section causes little or no lung tetanus. In such preparations stimulation of the peripheral end of the cut vagi also fails to influence the lung tonus. Poor physiological conditions in the frog are evidently associated with lung atony, just as these conditions usually involve atony and absence of stomach rhythm in all species of animals so far studied.

We have occasionally found preparations showing marked lung tonus before section of the vagi or destruction of the medulla, that is, a tonus greater than that of normal respiration. We are inclined to ascribe this to the following causes: *a*, temporary depression of the medulla or nervous "shock" due to cutting the spinal cord, strong mechanical stimulation of many sensory nerves; *b*, direct mechanical injury to the

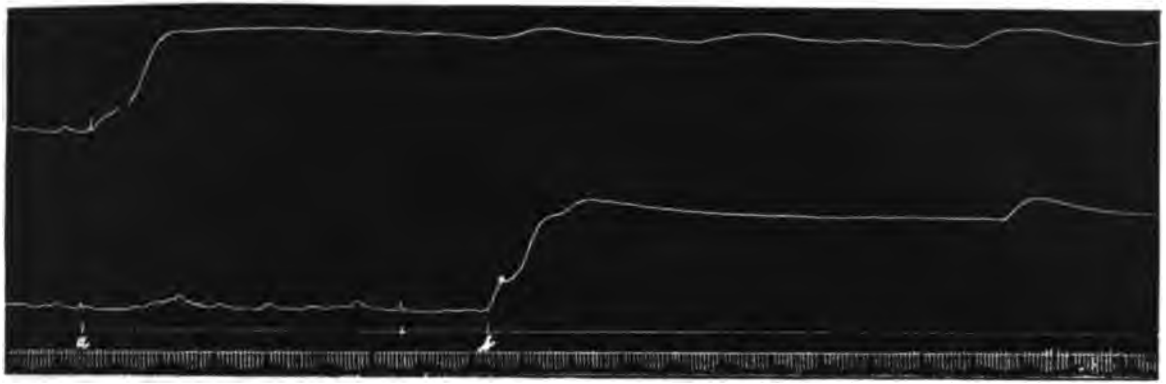

Fig. 6. Water manometer tracings of intrapulmonic pressure of frog (R. pipiens). Spinal cord transected and pithed below medulla. Frog fixed on dorsal side, abdomen laid open and lungs isolated from influence of skeletal muscles. Cannulae in tips of lungs. Glottis closed by forceps, shutting off at the same time air connections between the two lungs without interfering with lung circulation and lung innervation. *a*, Ligation of base of left lung (upper tracing). *b*, Section of right vagus (lower tracing = right lung). Signal line = base line pressure for right lung. Time, 5 seconds. Showing prolonged tetanus or tonus of lung neuro-musculature mechanism on severance of vagi nerves.

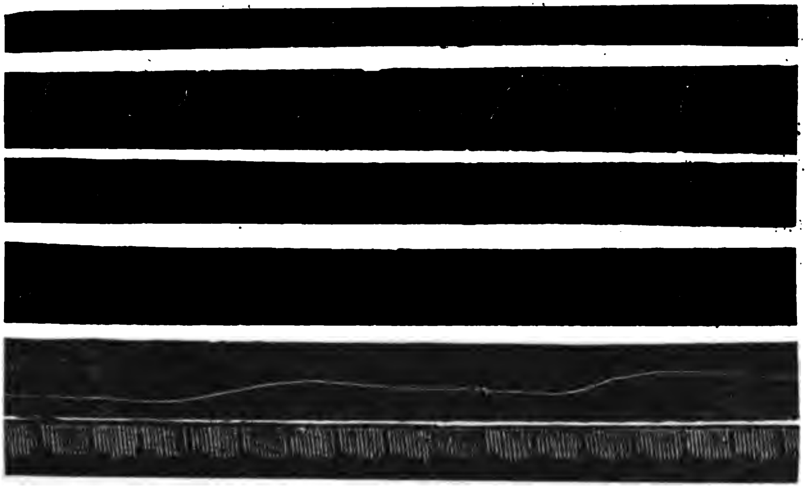

Fig. 7. Water manometer tracing of the intrapulmonic pressure in the frog (R. pipiens). Showing various types of peripheral automatism after isolation of the lungs from the central nervous system (section vago-sympathetic nerves or pithing of brain). Time, 5 seconds.

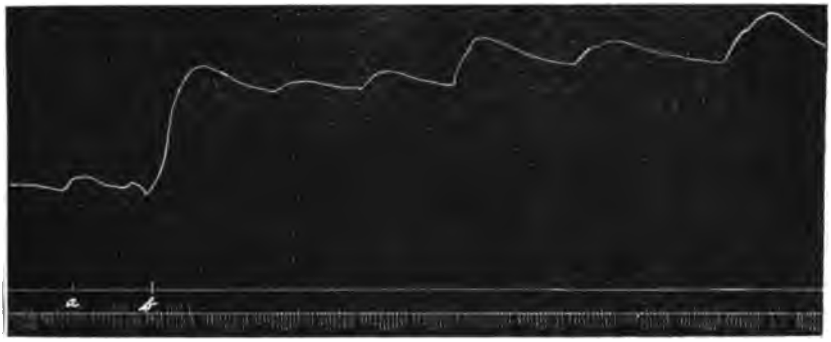

Fig. 8. Water manometer tracing of the intrapulmonic pressure in the frog's lung (R. pipiens), showing the incomplete tetany of the lung following destruction of the brain. Spinal cord cut and pithed below the medulla. Abdomen opened and lungs freed from influence of skeletal muscles. Cannula in tip of lung. Glottis closed by forceps, leaving vagi nerves and lung circulation intact. *a*, Transection of upper jaw. *b*, Crushing of brain (including medulla) by artery forceps. Time, 5 seconds.

lungs; *c*, partial asphyxia of medulla and lungs from failure of the circulation (unavoidable hemorrhage, etc.). In such preparations the section of the vagi or the destruction of the brain may cause a slight increase of the lung tonus (fig. 9). The lung of the bull frog passes into prolonged hypertonus on direct mechanical stimulation more readily than does the lung of the common grass frog (R. pipiens).

In our most vigorous preparation the lung tetanus following isolation from the central nervous system is extreme, that is, all of the air is driven out of the lung cavity into the water manometer and the lung cavity is completely obliterated. Even the traces of mucus are forced into the cannula in the tip of the lungs. The maximum intrapulmonic pressure thus developed is from 6 to 10 cm. of water above the pressure existing during normal respiration, that is, a total pressure of from 7 to 12 cm. of water. This is not the maximum pressure the lung tetanus is capable of developing. If the lungs are connected with a mercury manometer so that some air still remains in the lung cavity, even under the maximum lung tetanus following vagi section, the intrapulmonic pressure rises to the surprising height of 20 to 40 mm. Hg. (25–50 cm. water).

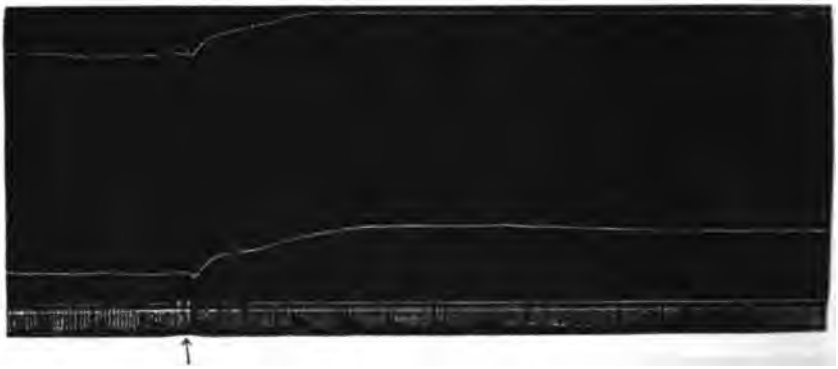

Fig. 9. Water manometer record of the intrapulmonic pressure in the frog's lung, showing moderate tetanus or tonus of the lung on destruction of the brain. Spinal cord cut and pithed below the medulla; frog fixed on dorsal side, and lungs isolated from influence of skeletal musculature. Cannula in tip of lungs; glottis closed by forceps, also shutting off air communication between the two lungs. Signal = crushing of brain with forceps. Signal line = the zero line of water pressure for right lung (lower tracing). Time, 5 seconds.

2. *The nature of the peripheral lung tetanus.* In most of our preparations the water manometer tracings of the lung tonus following isolation

of the lungs from the central nervous system show a straight line indicating a continuous tonic or complete tetanic contraction (fig. 9). The more vigorous preparations exhibit various types of rhythmic contractions superimposed on the continuous hypertonus, at least during the first 15 to 60 minutes following the lung isolation. But even in these preparations the rhythm fails before the complete failure of the tonic state of contraction in later stages of the record. It would thus seem that the appearance of rhythm on the hypertonic state of the lung is a question of physiological condition of the lung motor mechanism.

The usual type of the rhythmic lung contractions is shown in figures 6 and 8, that is, a slow rhythm, the contraction and relaxation requiring 2 to 3 minutes. In general, the more vigorous the preparation the more rapid the rhythmic contractions. In other preparations contractions last only 20 to 30 seconds, and occasionally this faster rhythm may be superimposed on the slower rhythm (fig. 7), both rhythms being in turn superimposed on the continuous hypertonus. Whether these varying types of contractions involve different musculatures cannot be made out by the present method of experimentation. The continuous hypertonus as well as the strong slow rhythm seem to involve the whole lung. The more rapid rhythm is too feeble to be detected by direct inspection of the lung. Nothing that could be interpreted as peristaltic contractions similar to those exhibited by other visceral structures has so far been noted by us, although our water manometer tracings of the lung hypertonus as slow rhythmic contractions suggest many points of similarity to the contractions of the empty stomach as recorded by the balloon method. This may be of significance in connection with the fact that the lung is a diverticulum from the foregut (esophagus).

It appears that this striking lung tetanus or hypertonus following isolation of the lungs from the central nervous system has not been observed by previous investigators, evidently because none have sectioned the vagi after closing the glottis under conditions permitting recording the neuro-muscular tonus of the lungs. Several workers have studied the influence of vagi section on the external respiratory movements of the frog. Mochi states that the lungs remain permanently collapsed and empty when the medulla and vagi are left intact but all the brain anterior to the medulla removed. This is probably an error. Langendorff claims, at least, that the external respiratory movements persist after removal of the brain anterior to the medulla. According to Martin (16) and Mochi (17), (18), (19), stimulation of the optic

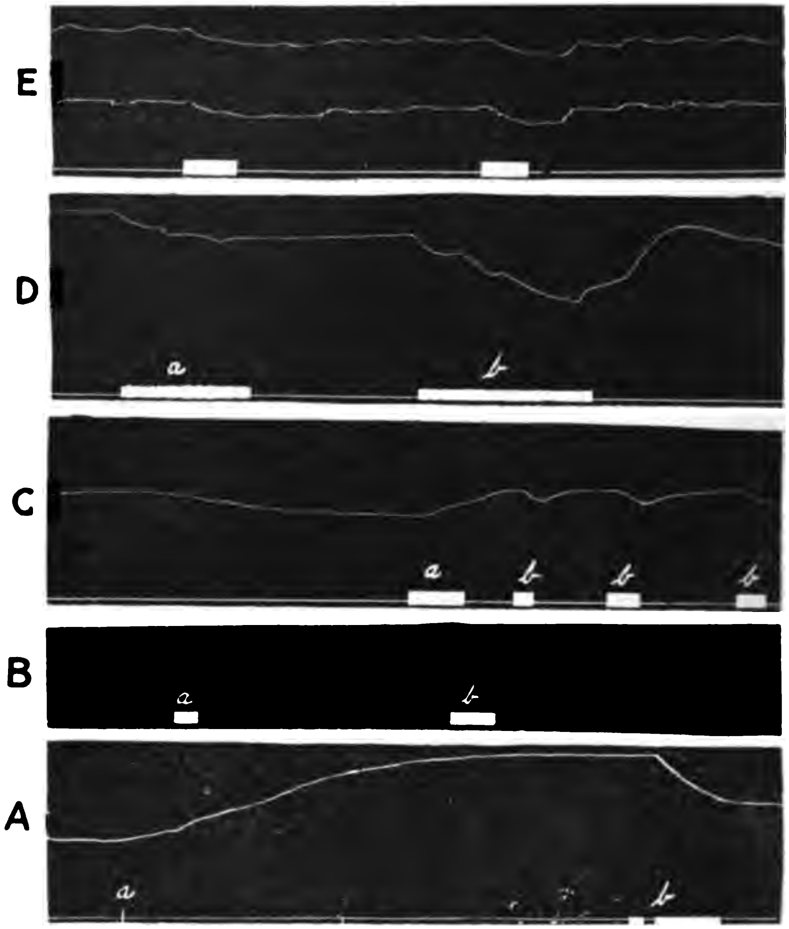

Fig. 10. Water manometer tracings of the intrapulmonic pressure in the frog (R. pipiens). Spinal cord cut and destroyed below medulla; lungs isolated and cannula fixed in tip of lungs. Glottis closed.

A: Right lung: *a*, section of vago-sympathetic nerve; *b*, stimulation of peripheral end of cut nerve with moderately strong tetanizing current.

B: Left lung: left vago-sympathetic nerve sectioned. Stimulation of peripheral end of cut nerve with weak, *a*, and strong, *b*, tetanizing current.

C: Left lung, after section of left vago-sympathetic stimulation of peripheral end of cut nerve with very strong, *a*, and moderately strong, *b*, tetanizing current.

D: Left lung, in very strong tonus after section of left vago-sympathetic nerve. Stimulation of the peripheral end of the cut nerve with weak, *a*, and strong, *b*, tetanizing current.

E: Upper tracing = left lung; lower tracing = right lung. Signal = stimulation of the peripheral end of the cut nerves. Showing inhibitions of lung tonus and contractions on stimulation of the peripheral end of the vago-sympathetic nerve.

lobes accelerates the respiratory movements. This may indicate a subsidiary respiratory center anterior to the medulla. At any rate mere decerebration does not induce lung tetanus in our experience.

Martin (16) states that destruction of the brain and spinal cord leaves the lungs entirely empty of air, but he does not make out or recognize that this is due to a persistent lung tetanus. Babak (2) quotes a number of authors as having shown that after vagi section or lung extirpation the frogs swallow air, periodically, into the stomach, and the air may actually escape by the cloaca. Berti and Marzemin (4) state that section of the vagi peripheral to the superior laryngeal branch results in irregular attempts at lung respiration on elevation of the temperature. Nikolides (20), (21) states that vagi section slows the respiratory movements making them at the same time irregular and stronger. Heinemann (9), one of the earliest observers, states that section of both vagi leads in the course of several days to such abnormal filling of the lungs that some of the viscera are pushed out through the cloaca. But when he opened the abdomen of these frogs the lungs were found collapsed or only partly filled. It is possible that Heinemann's frogs actually swallowed air into the stomach and intestines because of persistently constricted lungs. Soprana (24) states that vagotomized frogs breathe slower and deeper, but die more quickly from asphyxia on elevation of the temperature. According to Pari (22), the vagotomized frog is unable to fill the lungs, the lungs remain collapsed for weeks, and the air is forced into the stomach. It is possible that Pari's permanently collapsed lungs were in reality in a constricted state. But the method of observation did not suffice to record the fact.

It is certain that the force of the air swallowing would fail to cause air to enter the lungs against the maximum state of lung contraction found by us after section of the vagi nerves. But we do not know how long this hypertonus persists in the surviving animal, as all our experiments to date have been crucial. And even if the hypertonus, tonus or tetanus remained as long as the frog continued in good condition, failure of lung respiration may soon operate to place the frog below par in general, in which case there may be depression of the peripheral lung automatism already noted by us in animals in poor condition. The process of physiological readjustment of the peripheral lung motor mechanism may also come into play, similar to the readjustment that gradually takes place in the case of the heart and the respiratory center in the medulla after vagi section.

3. The action of the vagi and the cervical sympathetic nerves on the lung motor mechanism. a. The cervical sympathetic nerves. Section of the cervical sympathetic before its union with the vagus has no effect on the lung tonus (fig. 11), in marked contrast to the effects produced by section of the combined vago-sympathetic nerve. Electrical stimulation of the peripheral end of the cut cervical sympathetic causes slight contraction of the lung on the same side. It is difficult to stimulate the cervical sympathetic nerves, under the conditions of our experiments, and at the same time avoid escape of the current to the vagus

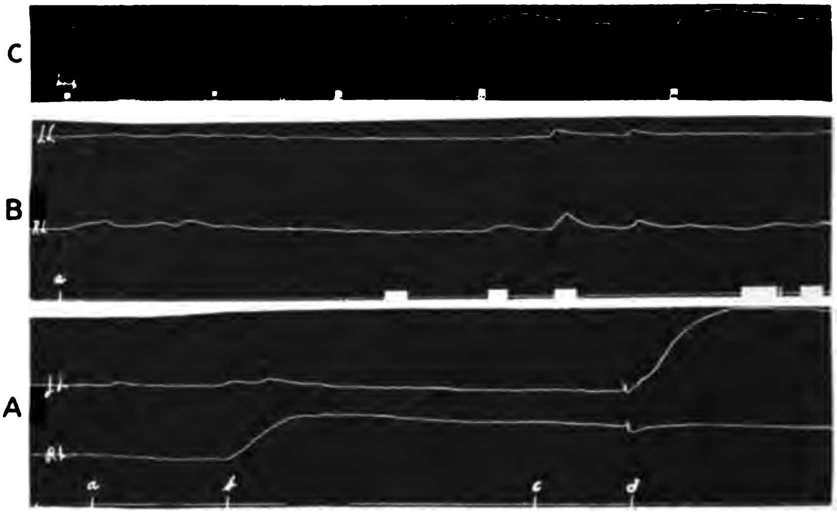

Fig. 11. Water manometer tracings of the intrapulmonic pressure in the frog's lungs (R. pipiens). Frogs decerebrated, animals fixed on dorsal side, lungs isolated from influence of skeletal muscle contraction. Cannula in tip of lungs. Glottis closed.

A: Upper tracing, left lung; lower, right lung. *a*, section right cervical sympathetic; *b*, section of right vagus; *c*, section of left cervical sympathetic; *d*, section of left vagus.

B: Upper racing, left lung; lower, right lung. *a*, section of right cervical sympathetic nerve; signal, stimulation of peripheral end of right cervical sympathetic. In this preparation the left cervical sympathetic and both vagi were intact.

C: Record from left lung, showing lung contractions on stimulation of the peripheral end of the cervical sympathetic nerve with strong tetanising current, the vagus being intact.

Showing motor fibers to the lungs in the cervical sympathetic nerve, but no effect on lung tonus from section of these motor fibers.

ganglion, or to other sensory nerves, thus inducing reflex effects. Our usual technique was to section the large brachial nerve close to the vertebral column, taking care not to injure the slender sympathetic trunk passing under it; also section the root of the hypoglossal, and after again sectioning these nerves peripherally, handle the cervical sympathetic by the stump of the brachial to which it is attached. We also made it a point to apply the fine pointed electrodes to the cervical sympathetic trunk at least 3 mm. distant from the vagus root. But even with the best of precautions escape of current to adjacent structures could not always be prevented. And we are inclined to explain the *bilateral* lung effects produced by the stimulation of one sympathetic (fig. 11, *B*) as due to escape, and consequent reflexes. It is to be noted further that it requires relatively strong tetanizing currents applied to the sympathetic trunk to secure the lung contractions. Inhibitory effects on the lung were never obtained from the cervical sympathetic nerves.

Our conclusion is that the cervical sympathetic trunk carries motor (but no inhibitory) fibers to the lungs via the vagi. Under the conditions of our experiments the section of these motor fibers has no effect on the lung tonus, showing that this motor mechanism is not in tonic activity, and that the section of the nerves is not a sufficient stimulus for even a transient contraction.

b. The vagi nerves. We have seen that section of the vagus induces permanent hypertonus in the lung of the same side. Stimulation of the peripheral end of the cut vagus with a tetanizing current causes an inhibition of this tonus followed by a return to the former state. The vagus stimulation is thus able to completely abolish (temporarily) the tonus induced by the vagi section. In the preparations showing no lung hypertonus on vagi section owing to peripheral lung atony vagus stimulation usually causes no lung inhibition.

In several such preparations we observed that the vagi also failed to influence the heart rhythm. We can state that the failure of the vagi to act in the normal manner on the lungs and heart in these preparations was not due to mechanical injury to the vagi or to the heart and lungs. The significance of this coincidence requires further investigation. It is well known to laboratory workers in physiology that one frequently encounters frogs in which the vagi stimulation fails to influence the heart. This inhibitory action of the vagus on the lung is obtained with the minimum and up to relatively strong tetanizing currents. The stronger stimuli produce at times motor after-effects, and very strong tetanizing currents may produce contraction only or a brief initial contraction followed by inhibition. This latter result was obtained by strong stimuli, especially in preparations showing less than the maximum lung tonus following vagus section.

Stimulation of the peripheral vagus inhibits not only the tonus but also the spontaneous rhythmic contractions that may be superimposed on the lung hypertonus following isolation from the central nervous system (fig. 10, *E*).

It is thus clear that the vagi and the cervical sympathetic nerves in the frog bear the same physiological relations to the lungs as they do to the heart, that is, motor fibers in the latter and inhibitory fibers in the former to both organs. We shall show later in the section on the action of drugs on the lungs, that some of the motor fibers to the lungs are true vagi fibers, and do not belong to the cervical sympathetic complex.

4. The peripheral lung automatism. We are now in position to analyze more definitely the origin of the motor hypertonus of the lungs after vagi section. It is not due to temporary stimulation of motor fibers. We have shown that section of the sympathetic nerve fibers has no effect. There are some motor fibers to the lungs of pure vagus origin. But cutting of these fibers produces no effect on the lungs, after previous paralysis of the inhibitory vagi fibers by large doses of nicotine. It is not due to mechanical trauma to the lungs. Ligation of the base of the lung may induce lung tetanus by direct trauma or by asphyxia, lung circulation being cut off. But stopping the circulation by excising the heart does not cause lung tetanus, and section of the vagi is done without touching the lungs or the adjacent structures. Moreover, the indirect mechanical disturbance of the pharynx and the base of the lungs is much greater from isolation of the vagi or the cervical sympathetic nerves, and these latter procedures do not bring on lung tetanus. The lung hypertonus is not an asphyxia phenomenon. Excessive lung ventilation, normal or artificial, will not prevent or abort it, if the vagi are sectioned or the brain destroyed. The lung hypermotility is not a temporary motor reflex state induced via the medulla by the powerful afferent impulses induced by the extensive operative trauma, for the lung is found collapsed and maximally contracted in frogs with the brain destroyed, without previous operative injury of any kind, and we know of no other reflex state that may persist for hours after lesion of the reflex path. It is not unlikely, however, that any condition inducing a peripheral lung hypertonus of a degree interfering with the respiratory functions of the lungs would augment the inhibitory action of the medulla on the lungs, probably through sensory fibers from the pulmonary branches of the vagi, a mechanism analogous to that of the depressor nerves and the cardio-inhibitory center.

In the experiments with pithing the medulla we at times obtained a slight temporary inhibition of the lung tonus prior to the typical lung tetanus. The temporary inhibition is evidently due to a transient stimulation of the medulla inhibitory center by the act of destruction. It is not probable that the subsequent lung hypertonus is due to a more lasting traumatic stimulation of the motor fibers, analogous to the effect produced on the heart by strong stimulation of the two sets of fibers in the vagi nerve trunks. This possibility is disproved by the fact that nicotine paralyzes the lung inhibitory nerve mechanism, leaving the motor nerve mechanism intact, and nicotine causes a lung tetanus which is not augmented by subsequent destruction of the medulla or section of the vagi.

Hence we must conclude that in the frog the vagi inhibitory fibers to the lungs are in constant or tonic activity, holding the peripheral motor automatism in check, and that on removal of this check the lungs go into a persistent tetanus or hypertonus. In other words, the lungs of the frog behave like the heart of many species of animals on section of the vagi; the heart beats faster, the lungs become hypertonic to a degree that nullifies their function.

These observations place the lung of the frog in the same category as the heart and the alimentary tract as regards independent peripheral motor automatism. In all these structures we have the same motor tissues, viz., nerve cells, nerve plexuses and musculature. We are therefore confronted by the same problems as regards the nature of the mechanism of the lung automatism that have engaged the attention of the physiologists in connection with the heart and the gut. A question of equal importance is the persistence or modification of this primitive lung automatism, in health and disease, in other groups of lunged animals.

CHANGES IN INTRAPULMONIC PRESSURE AS A RESULT OF THE STIMULATION OF VARIOUS AFFERENT NERVES

No investigations have been made on reflexes into the lung musculature. Most of the previous workers have been engaged in a study of the external respiratory phenomena of the frog. Wendenski (26) noted

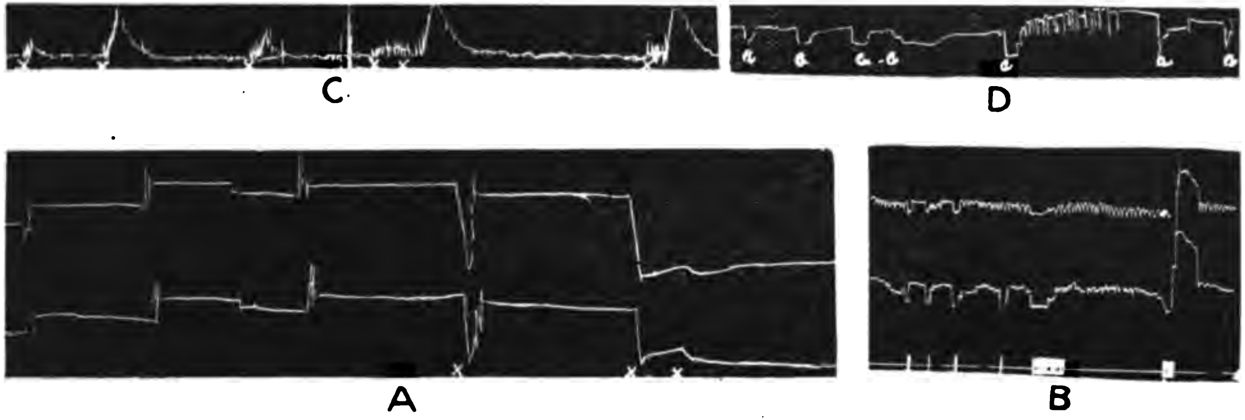

Fig. 12. Water manometer tracings of the intrapulmonic pressure in frogs. Glottis free. Cannula in tip of lungs. Tracings *A*, *B*, *C*, frogs decerebrated. Tracing D, spinal cord cut and destroyed below medulla leaving the brain intact. *A*, Bull frog. Upper tracing, left lung; lower, right lung. *X*, rubbing skin of hind leg.

B: (R. pipiens) upper record, left lung; lower, right lung. Signal, electrical stimulation of the urinary bladder.

C: *X* = gentle stroking of skin of hind leg, resulting in accelerated inspiration.

D: *a* = approach of finger on any moving object within the frog's visual field, followed by inhibition in a state of complete expiration or collapse of lungs.

"expiratory tetany" following weak stimulation of sensory fibers in the vagi. His method as well as that of other workers was not designed to note actual contractions of the lung itself, since in every case previous experimenters worked with an open glottis. However, tracings 15 and 16 of this article, taken from doubly vagotomized frogs, show very slight inspiratory and expiratory excursions of the flanks and long tonus variations obviously due to the strong tonus of the lung and the tonus contractions in the lung as seen by us.

Sensory stimulation of any sort, be it electrical or mechanical, has a powerful effect on the external respiration of the frog by either reducing the intrapulmonic pressure by reflex opening of the glottis, or if the latter is closed by hemostat or pressure by vaselinized cotton at the time of application of stimulus, by reflex lung contraction which will cause the intrapulmonic pressure to rise.

Figure 12 *A* shows at *X* the sudden opening of the glottis in decerebrated bull frog, holding air under considerable pressure, following gentle stroking of the skin of the hind leg. The first part of the tracing shows voluntary respirations (swallowing of air) with maintenance of high intrapulmonic pressure. The prompt collapse of the lung is followed immediately by marked respiratory effects which raised the intrapulmonic pressure to its original level. Stimulation of the skin in another preparation similarly prepared (fig. 12 *C*) not only increased the volume of the respiratory gulps, which were occurring regularly and continuously prior to the stimulation, but induced the animal in every instance to fill the lungs to the maximum capacity in the fashion described in the second section of this paper.

Figure 12 *B* shows a similar collapse of the lungs in Rana pipiens due to opening of the glottis following electrical stimulation of the urinary bladder with a moderately strong tetanizing current.

Tracing *D*, figure 12, was obtained from a frog with brain intact. In this animal the simple approach of the finger or person at *a* led to collapsed lung followed by more or less marked efforts at refilling.

In all tracings reproduced in figure 12 the glottis was open. These preparations, therefore, were not favorable for a study of the pulmonary activity itself (lung contractions or inhibitions) following the stimulation of various afferent nerves. In order to maintain the volume of air constant we closed the glottis by a mosquito forceps or vaselinized cotton and raised the intrapulmonary pressure to a point maintained by the animal under normal conditions and then noted the effect of the stimulation of the afferent nerves.

The ligation of the vagus on one side (mechanical stimulation) in many instances induced reflex contraction of the opposite lung. In the present state of our knowledge it is impossible to state whether record of such a contraction as shown in figure 13 *A* at *X* is due to reflex stimulation of the lung through the motor fibers of the vagus or due to a temporary inhibition of the tonic inhibitory control over the lung via the vagi, leaving the peripheral automatic mechanism in the lung unchecked. A possible answer to this question might be obtained by noting the effect of such stimulation in animals in which the tonic inhibitory mechanism has been previously paralyzed by nicotine. If under these conditions stimulation of the sensory nerves yields the same results the recorded contraction is the result, not of a temporary inhibition of the tonic inhibitory mechanism, but due to the reflex stimulation of the pulmonary motor fibers through the vagi (and sympathetics). Consideration of the law of reciprocal innervation would suggest that under normal conditions both mechanisms are involved in the phenomenon whose graphic record is that of a rise in intrapulmonic pressure.

Irrespective of the mechanism or mechanisms involved in ultimately effecting contractions of the lungs, we can confidently say that stimulation of the skin of the upper mandible, mild mechanical irritation of the anterior nares, mechanical stimulation of the bladder and cloaca, or electrical stimulation of the urinary bladder, mesentery, small intestine, pyloric end of stomach, esophagus and central end of the brachial nerve effect reflex contractions of the lung. These points are shown individually in figure 13 and figure 14, which with the accompanying legends are self-explanatory. The mode of preparation of the animals used in both series is virtually the same with this exception: In the preparations, records of which are illustrated in figure 13, the glottis was clamped by means of a mosquito forceps; in the tracings reproduced in figure 14 the glottis was kept occluded by pressure over it by a piece of vaselinized cotton.

Of these records two deserve particular attention. In figure 13, *D*, is recorded the powerful reflex lung contractions obtained by closure of the glottis by a hemostat. A decided inhibition preceded the contraction.

Tracing *E* in the same figure shows an unusually strong reflex contraction of both lungs as a result of crushing the skin of the lower mandible.

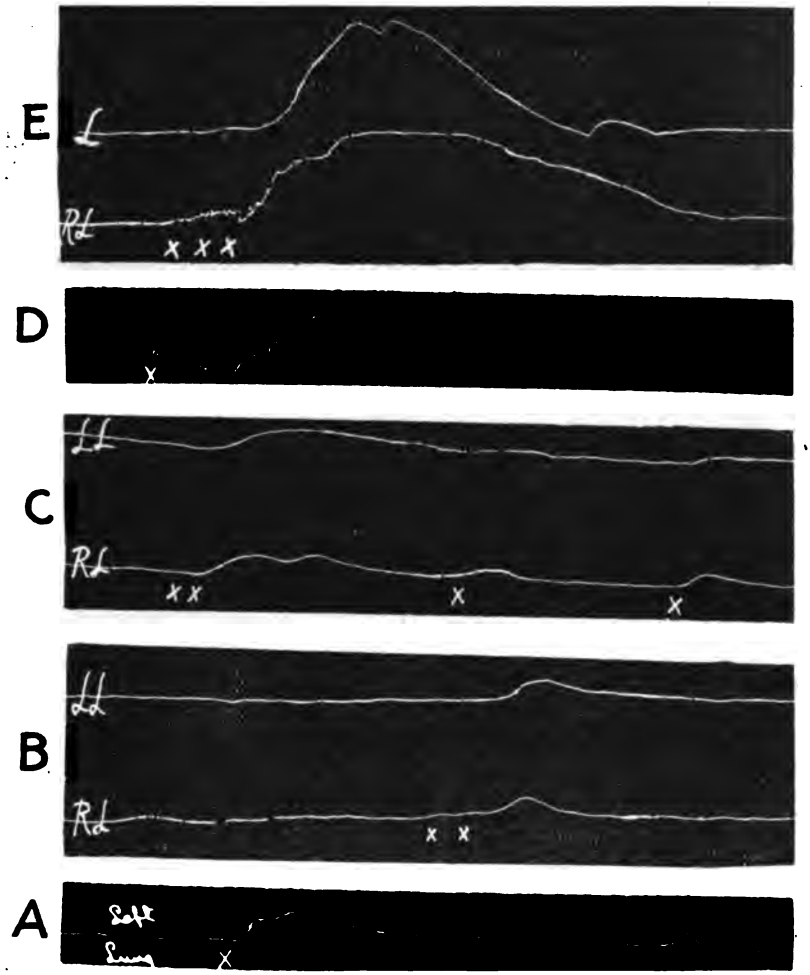

Fig. 13. Water manometer tracing of intrapulmonic pressure in the frog (R. pipiens). Spinal cord cut and destroyed below medulla. Cannula in tip of lungs, abdomen opened and the lungs isolated from abdominal and shoulder musculature. Glottis closed by clamp except in tracing *D.*

A: Left lung; *x*, ligation of right vago-sympathtic nerve, showing reflex contraction of left lung on vagus stimulation.

B: Upper tracing, left lung; lower, right lung. *X*, mechanical stimulation of the skin of the upper mandible, showing reflex lung contraction.

C Upper tracing, left lung; lower, right lung. *XX*, mechanical stimulation of the nares; *X*, mechanical stimulation of the cornea, showing reflex lung contractions.

D: *X*, closure of the glottis with artery forceps, showing temporary reflex inhibition of lung tonus followed by strong contraction.

E: Upper tracing, left lung; lower equals right lung. *XXX*, crushing skin of lower mandible, showing exceptionally strong reflex lung contractions.

In this series of experiments stimulation of the fundic end of the stomach (fig. 14, *D*, "*c*") with the electrical current yielded no reflex contraction of the lung at a time when stimulation of the pyloric end of the stomach and cardiac region of the esophagus with the same strength of current gave uniformly striking results.

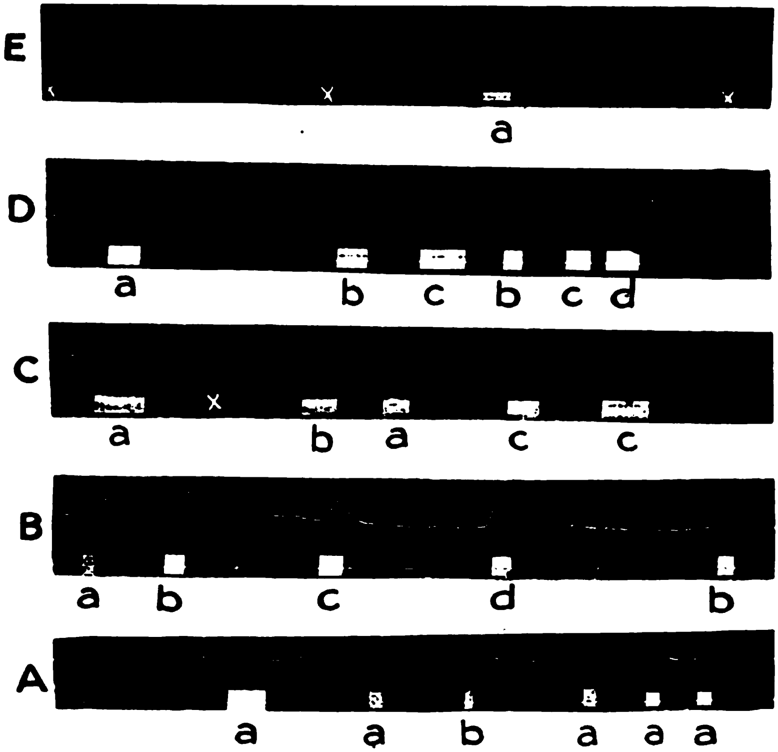

Fig. 14. Water manometer tracings of the intrapulmonic pressure in the frog's lung, showing reflex contractions of the lung musculature. Frogs decerebrated. Abdomen opened, lungs isolated from influence of skeletal muscle contractions. Cannula in tip of lungs, and glottis closed with a plug of vaselined cotton pushed into the pharynx.

A: *a*, mechanical stimulation (stroking) skin of hind leg; *b*, pinching toes of hind leg.

B: *a*, mechanical stimulation of urinary bladder; *b*, electrical stimulation of urinary bladder; *c*, mechanical stimulation of cloaca.

C: Electrical stimulation; *a*, large intestine; *b*, mesentery; *c*, small intestine.

D: Electrical stimulation; *a*, small intestine; *b*, pyloric end of stomach; *c*, fundus of stomach; *d*, esophagus (cardiac region).

E: *a*, electrical stimulation of central end of brachial nerve plexus. *X*, spontaneous respiration (quick up stroke).

In a summarizing sentence we might therefore state that the stimulation of every sensory nerve (afferent visceral or cutaneous) gives rise reflexly to lung contractions.

THE ACTION OF CERTAIN DRUGS ON THE MOTOR MECHANISM OF THE LUNG

Our interest in the action of drugs on the neuro-muscular mechanism of frog's lung had its inception during our study of the physiological action of the vagus on the lung musculature following electrical stimulation of the nerve. Such stimulation gave at outset variable results until we noted that the effects depended to some extent on the strength of the tetanizing current employed, as noted above. At any rate, we had good reason to suspect that the vagus carried both motor and inhibitory fibers to the lung motor mechanism. At this juncture it occurred to us that the use of drugs might be helpful in clarifying the situation.

Nicotine. Mindful of the action of nicotine in abolishing the inhibitory effect of vagus stimulation on the heart without affecting the motor action, we assumed that the drug might act similarly with respect to the inhibitory fibers in the vagus to the lung. If this were so, electrical stimulation of the vagus following the injection of nicotine might give clearer evidence of motor fibers in this nerve than before nicotinization. Since, furthermore, the paralysis of the ganglion cells in the course of the inhibitory fibers to the heart effected by this drug is preceded most commonly by *stimulation*, the same effect might be anticipated in the case of the inhibitory fibers to the lungs. If this were true nicotine ought to cause, on injection, an inhibition similar to, if not identical with, the inhibition of the heart commonly observed as the marked effect of stimulation of the vagus before the injection of the drug, especially if the tonic central inhibitory control exercised over the lungs via the vagi had been abolished by either sectioning of the nerves or destruction of the medulla.

The results obtained exceeded our expectations. Inspection of figure 15 (at *g*) shows that 1 mgm. nicotine when intravenously injected effects a pulmonary inhibition in the lungs released from the tonic inhibitory influence of vagus by section of these nerves (at *a* and *b*) which compares favorably with the inhibition obtained by previously stimulating the nerves with a tetanizing current of moderate intensity (see *e* and *f*).

If, on the other hand, the nicotine is injected intravenously in an animal following ligation and section of but one vagus, as in figure 16,

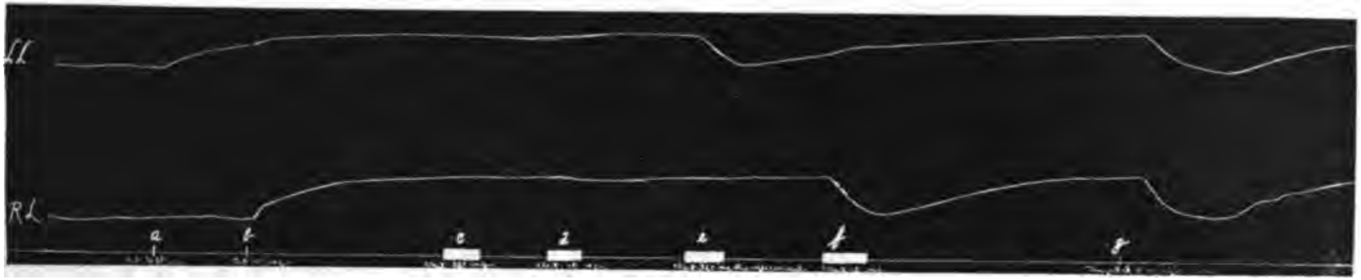

Fig. 15. Water manometer tracings of the intrapulmonic pressure in the frog's lung (R. pipiens). Spinal cord sectioned and destroyed below medulla. Cannula in tip of lungs. Lungs isolated from influence of skeletal musculature. Lower tracing, right lung; upper tracing, left lung. Glottis closed. *a*, Section of left vagus; *b*, section of right vagus; *c* and *d*, stimulation of left and right vagus with very weak tetanizing current; *e* and *f*, stimulation of left and right vagi with stronger tetanizing current; *g*, injection of 1 mgm. nicotine in 5 cc. Ringer's solution into abdominal vein.

Showing nicotine inhibitions of lung muscle tonus identical with the vagi inhibitions.

the immediate effect on the lungs is an inhibition of the tonus of the lung which has been denervated, and an escape from the tonic inhibitory influence exercised by the vagus on the lung which is still connected with its center, as at *c*, where the left lung (upper tracing) shows a rise in tonus occurring in the course of an inhibiton of the right lung, the latter comparing favorably with the inhibition effected by previous stimulation of the vagus (*b*). In this experiment the left vagus has been cut *physiologically* by the drug. If at the time of this drug cutting the vagus through central action is exercising its maximum inhibition on the lung, the effect of the primary stimulating action of the drug would not appear since the lung at the time of drug stimulation is already under maximum inhibitory control. As a matter of fact, in the majority of preparations this is apparently the case, the drug nicotine simply releasing the peripheral automatic mechanism from the maximum tonic inhibitory effect of the center through the vagus. This release is certainly complete for section of the vagus to this lung is without further effect on its tonus. This latter fact would furthermore indicate that the more or less prompt rise of intrapulmonary pressure following section of the vagus without nicotine was due, not to the mechanical stimulation of the motor fibers contained in this nerve, but to the removal of the tonic inhibitory control. That the vagus nerve contains such motor fibers can be shown very satisfactorily in any preparation that has been nicotinized. In figure 16 electrical stimulation of the nerves after nicotine (as at *g*, right vagus, and *h*, left vagus) gives now marked contraction of the lung instead of the usual inhibition before nicotine (*b*).

Figure 17, *A*, is offered as another example of this phenomenon. Following the release of the left lung from tonic inhibitory control exercised over it through central vagal control by section of the left vagus at *a*, 2 mgm. nicotine were injected at *b* with the result that the right lung was now released from its inhibition by the "cutting" action of the drug and the left lung was inhibited by the primary stimulation action of the drug on the inhibitory mechanism of the left lung. Figure 17, *B*, shows the change in effect as a result of electrical stimulation of the vagus nerve following the injection of nicotine. In this experiment stimulation of the vagus at *b* caused pronounced inhibition. The injection of nicotine at *c* was followed by the usual inhibition in the lung which has been released from the tonic inhibitory control by section of the nerve at *a*. Subsequent, however, to this nicotinization, electrical stimulation at *d* caused marked contraction of the lung instead of inhibition.

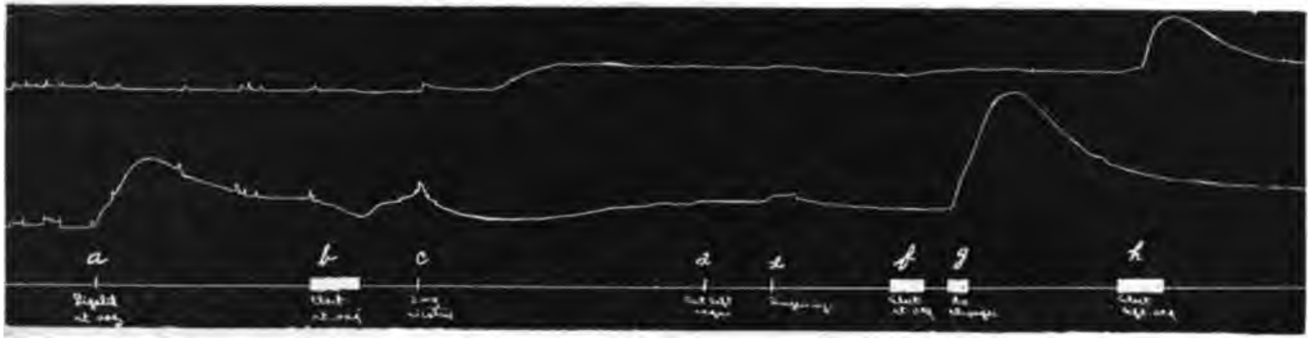

Fig. 16. Water manometer tracings of the intrapulmonic pressure in the frog's lung (R. pipiens). Spinal cord cut and destroyed below medulla; glottis closed; cannula in tip of lungs. Lungs isolated from influence of skeletal musculature. The quick up-strokes on tracings to the left of *C* are due to movements of the larynx in the spontaneous respiratory movements. Upper tracing, left lung; lower, right lung. *a*, Ligation of right vagus; *b*, electrical stimulation of right vagus; *c*, injection of 2 mgm. nicotine in 10 cc. Ringer's solution into abdominal vein; *d*, ligation, left vagus; *e*, injection of 10 cc. Ringer's solution into abdominal vein; *f* and *g*, stimulation of right vagus with weak and strong tetanising current; *h*, stimulation of left vagus with tetanizing current.

These tracings show the primary inhibitory action of vagus stimulation and nicotine on the lung hypertonus following section of the vagi, the paralysis of inhibitory fibers of the vagi by nicotine thus permitting the full development of peripheral lung tonus, and the reversal of the inhibitory action of the vagosympathetic action after nicotine.

The results are uniformly clear-cut and decisive. By means of this drug nicotine it is possible to differentiate between two types of efferent pulmonary fibers occurring in the vagus, i.e., inhibitory fibers and motor fibers.. The former exercise in the normal frog a powerful inhibitory control over the lung and are either more numerous or more readily susceptible to electrical stimulation than the motor fibers. It is only after the abolition of the inhibitory control of the lung by nicotine that

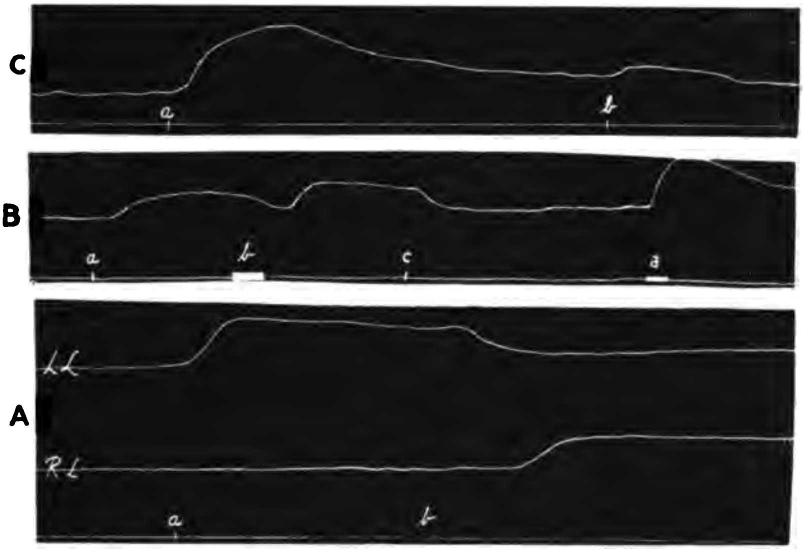

Fig. 17. Water manometer tracings of the intrapulmonic pressure in the frog (R. pipiens). Spinal cord cut and destroyed below the medulla. Glottis closed. Cannula in tip of lungs. Lungs isolated from influence by skeletal musculature.

A: Lower tracing, right lung; upper, left lung. *a*, Ligation of left vagus; *b*, injection of 2 mgm. nicotine in 10 cc. Ringer's solution into abdominal vein. Showing abolition of the tonic vagus inhibition of the lung neuro-muscular mechanism by nicotine.

B: Tracing from right lung. *a*, Ligation of right vagus; *b*, stimulation of peripheral end of right vagus with weak tetanizing current; *c*, injection of 2 mgm. nicotine in 10 cc. Ringer's solution into abdominal vein; *d*, stimulation of peripheral end of right vagus with same strength of tetanizing current as at *b*. Showing inhibition of lung tonus and paralysis of the vagi inhibitory fibers by nicotine.

C: Tracing from left lung. *a*, Injection of 5 mgm. nicotine in 5 cc. of Ringer's solution into the heart; *b*, injection of 1 cc. 1–1000 histamine into the heart. Showing stimulation of the lung by large doses of nicotine and stimulation by histamine after paralysis of the inhibitory nerves by nicotine.

electrical stimulation yields a more or less marked motor effect. Nor are these motor fibers of sympathetic origin running in the trunk of the vagus; for, granting the presence of some motor fibers in this nerve to the lung, the motor response on stimulation of the sympathetic after nicotine is smaller in a given animal than the response from the vagus itself, as noted earlier in the paper. In short, the vagus nerve contains two sets of fibers to the pulmonary motor mechanism of which the inhibitory exerts a tonic predominant control; the motor fibers are apparently not in a state of tonic activity for sectioning of the vagus after cutting the inhibitory fibers in this nerve by nicotine has no further effect on the intrapulmonic pressure. It would appear on the basis of our pharmacological studies that the inhibitory fibers of the vagus have interpolated in their course to the automatic tissues nerve cells on which the drug acts; the motor fibers on the other hand run directly to the automatic tissue.

Large doses of nicotine. Whereas the constant effect of the intravenous injection of small doses (2 mgm.) of nicotine in the previously denervated lung (by vagotomy) is inhibition, injection of large doses (5 mgm. or more) causes pronounced contraction of the lung. This is well shown in figure 17, *C*, where the injection of 5 mgm. caused more or less abrupt contraction followed by slow relaxation. It is probable that the nicotine in this dosage acts as a direct stimulant to the smooth musculature.

Atropine. This drug, even when given in large doses, does not paralyze the endings of the inhibitory fibers to the lungs as it paralyzes the cardio-inhibitory nerve endings. Figure 18, *A*, is a tracing from a frog which had received 1 cc. of a 0.1 per cent solution of atropine sulfate 45 minutes previous to experimentation. Pithing of the brain at *a* was followed by the typical escape of the lung from tonic central inhibitory control. The failure of atropine to paralyze the inhibitory nerve ending in the lung is shown further by the fact that even mechanical stimulation of the nerve at *b* gave powerful inhibition, as did electrical stimulation at *c*. As an after-effect of mechanical or electrical stimulation of the nerve there were pronounced motor effects. The results obtained from the right lung of another frog were somewhat different. Here (fig. 17, *B*) ligation of the vagus caused the usual escape of the lung from the inhibition. On the other hand, mechanical stimulation due to handling of the vagus nerve (at *b*) was followed by contraction. Stimulation of this nerve at *c* with a mild tetanizing current was in this instance followed by contraction instead of the usual inhibition.

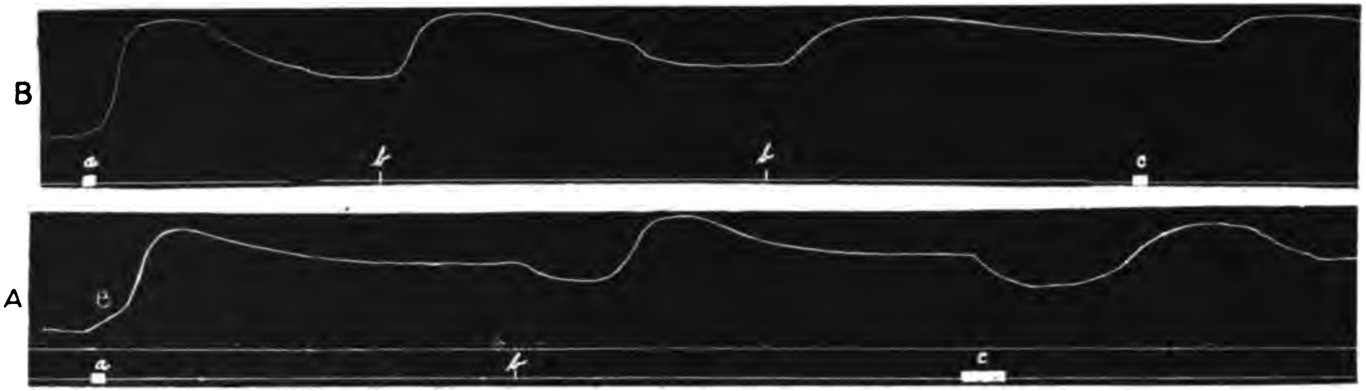

Fig. 18. Water manometer tracings of the intrapulmonic pressure in the frog (R. pipiens). Spinal cord cut and destroyed below medulla. Cannula in tip of lungs. Glottis closed. Lungs isolated from influence of skeletal muscle contractions.

One cubic centimeter of 0.1 per cent solution of atropin sulphate injected into the dorsal lymph sac 45 minutes before animals were prepared for the experiments.

A: Left lung; *a*, brain pith; *b*, mechanical stimulation (pulling) of left vagus; *c*, electrical stimulation of left vagus.

B: Right lung; *a*, ligation right vagus; *b*, mechanical stimulation of right vagus; *c*, electrical stimulation of right vagus with weak tetanizing current. Showing persistence of vagi inhibitory influence on the lung with exaggeration of the motor after effects after atropin in sufficient quantities to paralyze the cardiac vagi fibers.

To meet the objection that in these cases the dosage was too small and that the effects of the drug had worn off before experimentation was begun, we prepared another frog as follows: After decerebration, insertion of the cannula into the tips of both lungs, and clamping of the glottis, we sectioned the left vagus and obtained the usual escape of the lung from the tonic central inhibition control. Stimulation of this nerve gave the usual inhibition. The intravenous injection of ½ mgm. atropine did not effect the result of stimulation of the vagus. We now injected within 1 hour's time in successive doses, 1, 2 and 4 mgm. atropine sulphate. These injections did not change the usual reaction obtained from stimulation of the peripheral vagus. Following the injection of the last 4 mgm., the right lung escaped from central inhibitory control in a manner indistinguishable from section of its vagus nerve. Apparently this huge dose of atropine paralyzed the center, for section of the right vagus was without further effect. Stimulation of its peripheral end, however, yielded even now inhibition followed by contraction of the lung, indicating that the chief action on the lung of atropine in huge doses is not peripheral. Since decided motor after-effects result from stimulation of vagus to the lung in a heavily atropinized frog, we might conclude that the drug likewise has *some effect* in paralyzing the inhibitory nerve endings unless we assume that it sensitizes the motor nerve endings in the vagus. At any rate the peripheral action of atropine and nicotine are quantitatively decidedly different if one compares the dosage in milligrams and the results effected thereby.

Both drugs paralyze the center and likewise act on the peripheral mechanism. Nicotine accomplishes both results quickly and decisively in smaller doses, while atropine acts on the center only in large doses and only renders paretic the inhibitory terminations of the vagus. The frog is apparently quite tolerant to atropine. Eight milligrams injected intravenously into a decerebrated frog at one time suspends external respiration promptly (as does 1 mgm. of nicotine). Examination of the lungs shows them contracted. But almost complete recovery sets in within an hour and at this time the lungs assume their normal size and function.

Atropine in this dosage does not paralyze the terminals of the inhibitory fibers of the lung as it does the vagal nerve endings in the heart. In all of these atropinized preparations stimulation of the vagus nerve was without effect on the heart.

Adrenalin. In the frog the irrigation of the lung itself or injection of adrenalin chloride into the circulation causes inhibition of the automatic quick rhythm of the lungs which appears spontaneously as noted in a previous section of this paper or inhibits the hypertonic activity of the lung following section of the vagus. As illustrations of this effect of adrenalin we offer figure 19, *A* and *B*. The former tracing shows inhibition of the quick rhythm when adrenalin was applied to the lung directly at *X*; the latter, inhibition of the hypertonic state of the lung following vagotomy.

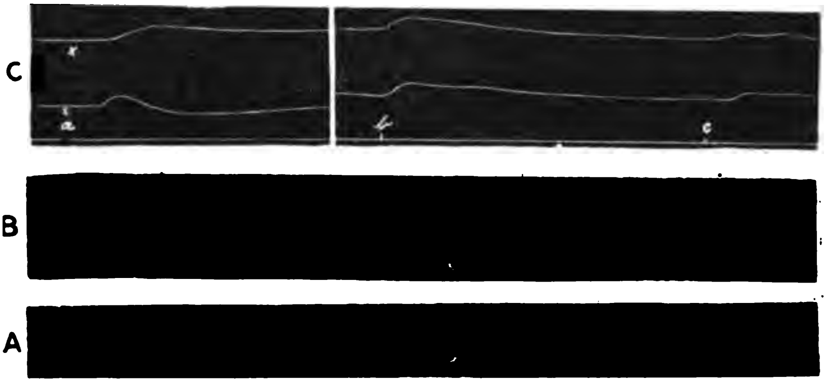

Fig. 19. Water manometer tracings of the intrapulmonic pressure in the frog (R. pipiens). Spinal cord cut and destroyed below medulla. Cannula in tip of lungs. Vagi nerves cut, and lungs isolated from influence of skeletal muscle contraction.

***A:* *X*, application of $\frac{1}{10}$ cc. adrenalin chloride (1–1000) in Ringer's solution to surface of lung.**

***B:* *X*, injection of $\frac{1}{10}$ cc. adrenalin chloride in 2 cc. Ringer's solution into the heart.**

***C:* Upper tracing, left lung; lower tracing, right lung. Intravenous injections of histamine in 2 cc. Ringer's solution; *a*, 0.01 cc.; *b*, 0.02 cc.; *c*, 0.06 cc. 1–1000 histamine hydrochloride.**

Showing inhibition of lung tonus by epinephrin and stimulation by histamine.

Histamine. Figure 19, *C*, and figure 17, *C* at *b* show the effect of histamine-HCl on the neuro-muscular apparatus of the lung when injected intravenously in varying concentrations. In moderately small or large doses it causes invariably a slight contraction of the lung which in amplitude bears no relationship to the dosage. Our experience, as a matter of fact, leads us to believe that successive doses of the drug given within a relatively short period of time have less and less effect because, possibly, of the cumulative poisonous property of this drug.

SUMMARY

1. The actual respiratory movements (opening of glottis and swallowing of air) are accompanied by relaxation of the tonus of the lung musculature, due either to greater action of the inhibitory fibers in the vagi or central inhibition of the motor nerve mechanism, on the assumption that the latter is in tonic activity. This inhibition occurs during the respiratory movements even when no air can enter or leave the lungs. It is therefore of central origin, an effect coördinated with the true respiratory act. From the point of view of utility the inhibition may be designated as a "receptive relaxation." The buccal movements that go on during the period between actual air swallowing do not seem to influence the lung tonus.

2. At the end of the respiratory act there is an active contraction of the lung musculature, after a latent period of 5 to 6 seconds. This contraction is of variable duration (10 to 20 seconds) depending on the respiratory rate and the condition of the lungs. The contraction is usually followed by a gradual tonus relaxation up to the next respiratory act. Occasionally this gradual tonus relaxation is absent. These active lung contractions are best seen during the pause of the Cheyne-Stokes type of breathing, which appears to be normal for the frog. The contraction is cut short by the next swallowing act, so that when the animal is breathing rapidly, the active lung contractions are not in evidence, the lung musculature being in a continuous state of "receptive relaxation." The active lung contractions following a respiratory act can be accounted for by a lowering of the inhibitory influence, thus permitting the peripheral automatism to come into greater play. We have not been able to determine whether motor innervation via vagi and sympathetic nerves also play a rôle as contractions follow the respiratory act even when no air enters or leaves the lungs. It is not a reflex initiated by the stimulation of pulmonary sensory fibers through lung distention. It is probably entirely central in origin and referable to the respiratory center, the inspiratory discharge of this center having the immediate effect of a temporary stimulation of the inhibitory mechanism for the lung tonus, the lung contraction of the end of the inspiration merely signifying excess back swing of the central inhibitory control on its return to the more or less constant level.

3. Section of the cervical sympathetic fibers has no effect on the lung tonus but stimulation of these fibers before they join the vagi

nerves causes lung contractions. The cervical sympathetic nerves contain a few motor fibers, but no inhibitory fibers to the lungs. These motor fibers in the sympathetic do not appear to be in tonic activity, but our experiments on this point are not final.

4. Section of the vagi nerves or destruction of the medulla causes a permanent hypertonus or incomplete tetanus of the lungs. This is due to removal of a tonic inhibitory check on the peripheral lung motor mechanism (peripheral neuro-muscular automatism). Ligation of the pulmonary branches of the vagi produces the same effect. The only part of the central nervous system necessary for this tonic inhibitory control is the medulla. Section and destruction of the entire spinal cord below the medulla has no permanent effect on the tonus mechanism. Decerebration has likewise no permanent effect on it. Destruction of the midbrain causes a temporary diminution of the lung inhibition probably through a "shock" state of the medulla. The afferent aspect of this tonic lung inhibition requires further study. The most important afferent pathway is probably the pulmonary branches of the vagi.

The contractions of the lungs following vagi section are powerful enough to develop a pressure of from 20 to 40 mm. Hg., and if the glottis is not artificially closed all the air in the lung cavity is forced out, the lungs contract down to a solid mass and are thus rendered useless as organs of respiration. All our data on this point are those of acute experiments lasting only 2 to 3 hours. The possible readjustment of this peripheral lung automatism to meet the needs of the animal after double vagotomy is being investigated by long time experiments.

5. Stimulation of the peripheral end of the vagi inhibits the lung tonus induced by vagi section. Strong tetanizing currents applied to the peripheral vagus trunk usually cause strong contractions following the primary inhibition. Nicotine paralyzes the lung inhibitory fibers of the vagi apparently without injury to the motor fibers, so that after nicotine vagus stimulation causes lung contractions only. These contractions are stronger than can be caused by stimulation of the cervical sympathetic nerve. Hence, on the basis of the usual interpretation of nervous action, the vagi carry both inhibitory and motor fibers to the lungs, the former predominating and being tonically active like the cardio-inhibitory mechanism in many animals.

By stimulation of the peripheral end of the vagus we have so far failed to cause a greater tonus relaxation in the lungs than that which existed before vagi section. This means either that the tonic vagi inhibitory action is ordinarily maximal, or that the simultaneous stimulation of the motor fibers in the vagi trunks neutralizes a part of the inhibitory effects.

The efferent actions of the vagi and the cervical sympathetic nerves on the lungs are unilateral. Only occasionally have we seen effects on the lung of the opposite, in case of sympathetic stimulation. This was probably due to escape of current, and not to actual nerve crossing.

6. Reflex contraction of the lungs are induced by the stimulation of the afferent fibers in the vagi, by stimulation of the cutaneous nerves, the sensory fibers in the nares and the cornea, and the sensory fibers in the visceral organs. These lung reflexes could be brought about either by augmented action of the motor nerve mechanism or by depression of the tonic inhibitory mechanism. On the basis of the usual conceptions of reciprocal innervation both factors are probably involved. It is thus clear that practically all afferent nerves have reflex connection with the medullary nuclei controlling the lung tonus and contractions.

7. As stated above, it is possible to differentiate between the motor and inhibitory fibers in the vagus trunk by means of nicotine. This drug in *moderate doses* (2 mgm.) paralyzes not only the respiratory center but also the peripheral inhibitory mechanism so that subsequent stimulation of the vagus causes more or less powerful lung contractions in place of the usual inhibition resulting from stimulation of this nerve before nicotinization. If nicotine is injected in a frog that has suffered bilateral vagotomy with the usual escape of the tonic inhibitory control of the corresponding lung, nicotine effects a marked inhibition of this lung and after a slight interval an escape from central control of the other lung. The escape of the one lung does not occur until the primary and temporary inhibition (stimulation) of the peripheral mechanism in the other is about over. This probably means that the lung still connected with the center before nicotinization is under maximal central inhibitory control since the drug in this instance produces no primary inhibition. Injection of nicotine in any case destroys the central tonic inhibitory control of the lungs similar to destruction of the medulla or double vagotomy. In large doses nicotine causes contraction of the lung musculature probably by direct stimulation of the muscular elements.

8. Atropine in doses large enough to paralyze the cardio-inhibitory fibers of the vagus has no effect on the terminations of inhibitory fibers in the lungs. In fact, even huge intravenous doses (8 mgm.) do not

completely paralyze these terminations. Such doses paralyze chiefly the medullary centers which send out the inhibitory impulses. As a result the lungs contract. But even this center recovers within an hour and again assumes its tonic inhibitory control over the lungs.

9. Histamine in small or large doses (0.01 to 0.07 cc. 1:1000 sol.) causes temporary contraction of the lung musculature.

10. Epinephrin inhibits both the peripheral automatic tonus and the peripheral automatic rhythm if one is present.

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THE EFFECT OF ADRENALIN ON VENOUS BLOOD PRESSURE

HELENE CONNET

From the Physiological Laboratory of the Johns Hopkins University

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A REVIEW OF THE LITERATURE ON VENOUS BLOOD PRESSURE

Regulation of venous pressure by: 1. Peripheral resistance. It is usually stated that, in general, increased peripheral resistance (in the arterioles)—other things being equal—causes a rise in arterial pressure and a fall in venous pressure and that decreased resistance has the opposite effect.

Bayliss and Starling (7) and Plumier (61) advance the idea that an increased peripheral resistance caused by vasoconstriction decreases the capacity of the vascular system and tends to cause a rise of pressure in all parts of the system. What change will occur in the venous pressure will depend upon whether the influence of the decreased flow from arteries to veins causing a fall, or the decreased capacity of the system causing a rise, predominates. The opposite state of affairs holds in case of decreased peripheral resistance. Sometimes the tendency for the venous pressure to rise is exactly counter-balanced by the tendency to fall. Bayliss and Starling (7) cite, as an illustration, the absence of venous pressure change when vasomotor paralysis has been induced by section of the cord just above the first thoracic segment. Plumier (61) also illustrates this point. He finds no change in venous pressure after weak or strong stimulation of the central stump of the vagus, both vagi being sectioned. In the case of weak stimulation, vasodilatation was produced while strong stimulation produced vasoconstriction. In each case, however, the influence of the peripheral resistance on the venous pressure was balanced by the opposite influence of the change in the capacity of the system.

According to Bayliss and Starling (7), sometimes the venous pressure-raising factor in vasoconstriction of the arterioles predominates, as when the splanchnics are directly stimulated or the vasomotor center is stimulated by asphyxia. The objections, as advanced by Hill and Barnard (37) and Plumier (61), to attributing this rise in venous pressure to decreased capacity of the vascular system will be discussed later.

2. Heart rate. The authors mentioned above (Bayliss and Starling, and Plumier) state that a rise in venous pressure is obtained when the heart slows or stops beating because there is a tendency toward equalization of pressure throughout the system, resulting in a fall in arterial and a rise in venous pressure due to the elasticity of the arteries forcing more blood into the veins. Usually in a slowly beating heart the output per minute decreases and hence the heart does not pump into the aorta per unit of time as much as it did before, thus causing a back pressure in the pulmonary veins, which eventually affects the right heart and causes a rise in the vena cava pressure. This would be true especially when there was an increased resistance to the blood-flow in the arteries, as in vasoconstriction.

Bayliss and Starling (7) consider that most of the rise of venous pressure after peripheral stimulation of the vagus is due to the decreased capacity of the system which comes as a result of the anemic stimulation of the vasomotor center following the low arterial pressure brought about by such stimulation. Their proof of this is that only a slight rise is occasioned by stimulation of the vagus when either the cord or the splanchnics are cut. Plumier (61) has a different explanation for this. He considers the slowing of the heart of primary importance and the vasoconstriction of secondary. He says that cutting the cord or splanchnics causes such a marked vasodilatation (arterial pressure in one of Bayliss and Starling's experiments fell from 120 mm. to 60 mm. Hg.) that tendency toward equalization of pressures cannot show the effect that it would, if the conditions of the vascular system were normal. This point is perhaps brought out more clearly by considering what changes take place in the vascular system under asphyxia, produced by removal of artificial respiration in an animal whose chest has been opened. Bayliss and Starling offer an explanation for this rise in venous pressure, similar to that given in connection with vagus stimulation. Plumier (61) attempts to prove his point that vasoconstriction is of only secondary importance, by comparing the effect of asphyxia before and after section of the vagi. With vagi intact the arterial pressure rises only slightly but the heart beat soon becomes very slow, and coincident with this slowing the venous pressure in the inferior vena cava and the external jugular rises markedly. As soon as artificial respiration is renewed and the heart beats faster, the venous pressure falls. In the experiment when both vagi are cut, the arterial pressure rises, due to vasoconstriction, but the venous pressure remains unchanged, until (100 seconds after the beginning of asphyxia) the heart becomes paralyzed and consequently slows, and the blood pressure falls. At this time the venous pressure rises. Furthermore, after artificial respiration has been renewed, the arterial pressure rises, due to continued vasoconstriction, and when it is at its maximum the venous pressure has already fallen to normal.

The difficulty here, it seems to me, is in trying to make either heart-slowing or vasoconstriction alone account for the rise in venous pressure. Bayliss and Starling (7) themselves, when discussing the rise of venous pressure after splanchnic stimulation, say (p. 172): "In experiment 5 one of the vagi was intact and the heart was slowed as usually occurs when the splanchnics are stimulated. One might be inclined to ascribe the rise of venous pressure to this slowing of the heart, were it not that in other experiments where both vagi were divided, we still obtained a slight rise on stimulation of the splanchnics." Evidently, then, the slowing of the heart is responsible for the greater part of the venous pressure rise, if not absolutely all. On the other hand, one cannot see how Plumier can be sure that Bayliss and Starling are wrong in ascribing some of their rise in venous pressure, after asphyxia for instance, to the decreased capacity of the system, since no experiment has been performed in which that factor has been ruled out, granting that Bayliss and Starling's attempt to rule it out, not only ruled it out, but introduced a new factor, that of vasodilatation after section of the cord or splanchnics, which vitiated the comparison. It would seen as though this point might be settled by an arrangement, such as that used by Heard and Brooks (31), for keeping the arterial pressure constant under varying experimental conditions. When, after adrenalin or asphyxia with vagi intact, the change in capacity of the system, due to vasoconstriction, was not allowed to be effective, one could then see how much this factor has to do with the rise in venous pressure on slowing of the heart.

3. "Respiratory pump." According to Hill and Barnard (37) none of the above explanations is adequate in accounting for the rise of venous pressure obtained as a result of various experimental procedures. They attribute the rise which occurs on arrest of the heart under peripheral vagal stimulation to the respiratory spasms produced, and on asphyxia to strong abdominal and general muscular movements, for in curarized animals these rises do not occur, at least in the case of asphyxia. Their curarization was sufficient to abolish natural respiration, but did not interfere with the heart or tone of the arterial system. As regards vagal stimulation, they say (p. 348), "we ourselves unfortunately have not been able to maintain arrest of the heart in the curarized animal for long enough time to settle this point." Yet in figure 12, page 342 of this article, they show a rise in venous pressure in the same animal on stimulation of the vagus before and after the administration of chloroform. "In the first instance the escape of the heart is complete, the respirations are greatly intensified and by the powerful expiratory spasms of the abdominal muscles the venous pressure is greatly raised. In the second case the inhibition is complete while the respirations, weakened by the chloroform, remain unaltered during the standstill of the heart." On examination of the second curve one sees the venous pressure beginning to rise as soon as the beat of the heart is stopped, but while it is rising the animal was changed to the feet-up position. The venous pressure continues to rise until the heart once more begins to beat. This rise of venous pressure evidently cannot be ascribed to the activity of the respiratory muscles since no change in respiration occurs during the standstill of the heart, nor, probably, to the feet-up position which was assumed during the rise of venous pressure.

These authors object to any explanation of these phenomena which assumes that a rise in venous pressure can be brought about by sending more blood into the venous system, as occurs on arrest of the heart, or by decreasing the capacity of the vascular system by arterial vasoconstriction. They believe that the vascular system is not filled to distention and that since the veins can hold all the blood of the body without distention, no increase in amount of blood in the veins or decrease in arterial caliber can cause a rise in venous pressure. To prove that the veins can hold all the blood of the body at zero pressure they cite the condition that exists in the animal after death. Then, since it might be thought that after death the tone of the vascular system had passed off, they attempt to prove their point in a living animal. After stopping the heart by vagus stimulation, they alternately placed the dog in the vertical feet-down and horizontal positions until all the blood had passed from the arteries into the veins, and past the venous valves so that no reversal of flow could take place. They say (p. 346), "In this experiment we produced a positive pressure in the veins and no pressure in the arteries. . . . . Since the arteries are emptied of blood the whole system is not filled to distention. . . . ." Just because the arteries are much more elastic than the veins and can, when the heart stops beating, empty themselves of blood and produce a greater positive pressure than before in the veins, it does not therefore follow, it seems to me, that the vascular system, while the heart was beating, was not filled to distention.

Furthermore, in refutation of Bayliss and Starling's belief that anemia of the brain in vagal arrest causes vasoconstriction, they say (p. 348), "In our experiments when the heart has escaped from arrest, there has not occurred any great rise of arterial pressure which we should expect to indicate vasoconstriction." Why should one expect any great rise of arterial pressure after the heart has begun to beat again? With the resumption of the heart beat and consequent rise of arterial pressure toward normal, the cause of the vasoconstriction, anemia of the brain, is removed.

"Any appreciable increase of vena cava pressure is due either to the reduction of the capacity of the venous system by the action of the respiratory muscles, or to the failure of the heart in maintaining the systolic output" (p. 350). By this, I presume, is meant the inability of a slowly beating heart to expel per minute as much blood as it receives per minute, thus causing a back pressure in the veins. I fail to see how this could cause a rise in venous pressure if their contention is correct, that the venous system is capable of holding all the blood of the body without distention, especially since it occurs almost immediately on slowing of the heart, before the arteries could have emptied the greater part of their blood into the veins. Of course, it may be contended that the cava is only a part of the venous system and it might become distended when a large amount of blood collected in it, but this hardly seems a valid contention in the case of the immediate rise in venous pressure on arrest of the heart.

4. Chemical mechanism. Roy and Brown (64) in experimenting with the frog's web, tongue and mesentery, found that temporary anemia was followed by dilatation of arteries, capillaries and veins. This dilatation they attribute to a "relative diminution in the lymph of certain of the constituents of the blood, or the presence in increased amount of certain of the products of tissue exchange, or both of these combined" (p. 359). This effect, they say, is independent of cerebrospinal vasomotor effects; it may possibly be due to action on peripheral vasomotor ganglia, but they think it is more probably due to direct action on vessel walls. These causes operate in other congestions and the authors feel that this automatic regulation of the peripheral circulation is of very great importance.

Henderson and Harvey (33), and Henderson (32) develop the idea of a peripheral chemical control of venous pressure "largely through variations in the CO_2 content of the venous blood." CO_2, by relaxing the veins, they believe, removes the resistance to the flow of blood from capillaries to veins, and so causes an increase in venous pressure. They speak of such a relaxation as though it were merely the complete or partial removal of a clip, allowing more blood to flow into a vessel whose caliber remains the same. But if relaxation means more than this; if it means an actual increase in the capacity of a portion of a blood vessel, due to the lessening of vascular tone, it is difficult to see how this relaxation can cause a rise in the pressure in the vessel even though there is an increased amount of blood present.

5. Nervous mechanism. In a study of the regulation of the blood supply of the brain in 1890, Roy and Sherrington (65) found that stimulation of the peripheral stump of the vago-sympathetic nerves in dogs produced sometimes a rise, sometimes a fall of general venous pressure. They think that this probably means that the vago-sympathetic trunk contains vasomotor fibers to the veins. As discussed in the section on heart rate, later observers have attributed this rise which peripheral vagus stimulation gives, to the slowing of the heart which is occasioned by such stimulation. Slowing of the heart due to paralysis from asphyxia with both vagi cut has been shown by Plumier to give a rise in venous pressure comparable to the rise caused by peripheral vagus stimulation. This perhaps does not prove that the vago-sympathetic nerves possess no venomotor fibers, but there seems to be perfectly adequate explanation for the venous pressure change without assuming the existence of such venomotor fibers. That Roy and Sherrington sometimes found that stimulation of the peripheral stump of the vago-sympathetic nerves produced a fall in general venous pressure, may possibly have been due to an escape of current to the central end of the nerve through the fluid medium surounding the tissues. One cannot tell what happened to the heart rate in these cases of a fall in venous pressure for no graphs are given or statement made in regard to it.

Thompson (72) in 1893 observed constriction of the superficial veins of the hind limb of dogs and rabbits on stimulation of the sciatic nerve or the spinal cord. The constriction took place in short sections, the diameter of the vein between the segments remaining unchanged. Bancroft (3) confirmed these observations and extended the work.

He observed the appearance of the veins of the hind limb of a cat (rabbits also were used but were not found nearly as satisfactory) before and after section of various nerves, and thus traced out the vasomotor supply. The cell body of the pre-ganglionic fiber lies in the spinal gray matter, the axis-cylinder emerging in the anterior root of the 1st, 2nd, 3rd or 4th lumbar nerve, following the corresponding white ramus into the sympathetic chain, running down it for a certain distance. The cell body of the post-ganglionic fiber lies in the 6th or 7th lumbar sympathetic ganglion, the axis-cylinder running to the veins in the sciatic nerve. Langley's nicotine method was used to determine the position of these ganglia.

The experiments of Gunn and Chavasse (30) and Crawford and Twombly (18) on the effect of epinephrin on isolated veins lend support to the belief that a venopressor nervous mechanism exists in the veins. The details of these experiments will be given later in this paper under the head of adrenalin.

Hooker (41), (42) has demonstrated veno-pressor fibers in a nerve trunk running from the inferior mesenteric ganglion to the veins of the large intestine. A rise in venous pressure in an isolated loop of intestine was induced by stimulation of: *a*, the nerve to the part (peripheral mechanism); *b*, a sensory nerve such as the saphenous (central reflex mechanism); *c*, the central mechanism by asphyxia. Section of the peripheral nerve or destruction of the medulla destroyed the reflex. A probable failure of this mechanism in shock is predicated by Morison and Hooker (55).

A contraction of the veins seems to be the cause of the increased capillary pressure found in the blue-handed type of irritable heart cases as described by Briscoe (9). This is especially illustrated in those patients whose hands were sometimes normal and sometimes blue. The average readings of this class, when the hands were normal, were: venous pressure, 10.6 cm. H_2O; capillary pressure 25.3 cm. H_2O; and when blue were: venous pressure, 10.6 cm. H_2O; capillary pressure, 33.3 cm. H_2O. Whether this contraction of the veins falls under the chemical mechanism theory or the nervous mechanism theory, the author does not state. One presumes, from the article, that it is the latter.

It is not intended in this paper to review in detail the literature on portal venous pressure, as it forms a rather specialized type of venous pressure. A comprehensive review of the literature which has established the presence of a vasomotor mechanism in the radicles of the portal vein, is to be found in Burton-Opitz's (13) and Edmunds' (22) papers.

Effect on venous pressure of: 1. Adrenalin. a. Effect on isolated veins. Dunn and Chavasse (30) have tested the effect of adrenalin (1 to 100,000) on isolated ring preparations from various veins in the sheep (external jugular, mesenteric, superior and inferior cava) and find a constriction similar to that which occurs in the arteries. The external jugular gave a greater response than the superior and inferior cavae. The amount of response of the mesenteric vein could not be compared with that of the other veins, for the temperature in the case of the mesenteric was 41°C., while in the other experiments it was 36°C. From this they judge that the veins probably contain venoconstrictor nerve fibers from the thoracico-lumbar sympathetic nervous system. Crawford and Twombly (18) corroborate these observations, finding constriction of ring preparations from femoral, iliac and saphenous veins of the dog. Their work on roosters is interesting in that they find some veins that contract in adrenalin solutions, and others that do not. Rings of the jugular vein of white Leghorn roosters, taken from the middle of the neck, contract slowly with 1 to 60,000 adrenalin in oxygenated Ringer's solution, but rings from the jugular vein near the head and from the large vein of the wattles gave no response. This argues, they feel, against a vasomotor supply to the cephalic end of the jugular vein and the veins of the wattles, and they suggest that perhaps the absence of vasoconstrictor fibers in wattles may be one of the reasons why they blue so easily.

b. Effect of intravenous injections. Hill (36) found that intravenous injection of suprarenal extract into a dog whose vagi had been divided caused a rise in arterial pressure of 1170 mm. $MgSO_4$ solution, while the vena cava pressure remained unaltered. Plumier (61) attributed the rise in superior and inferior vena cava pressure, which he obtained on intravenous injection of adrenalin in the intact animal (dog), to the slowing of the heart which such an injection occasions. He feels that one does not get as great a rise with say a 0.4 mgm. injection as the slowing of the heart would seem to indicate, but this may be explained by the fact that the increased force of the heart beat tends to reduce the venous pressure. However, after cutting the vagi, unless a very large dose is administered, there is no change or only a slight rise in venous pressure. In both cases there was important vasoconstriction resulting in a considerable rise in arterial pressure, but this decreasing of the capacity of the vascular system, he points out, is not sufficient, in itself, to change the venous pressure. Capps and Matthews (16) find that a small dose (they do not state the amount) of adrenalin does not affect the venous pressure, but a large dose, such as ⅛ mgm. (2 minims) of 1 to 1000, causes a rise of from 10 to 80 mm., and that coincident with this rise, the heart is markedly slowed. "The rise in venous pressure was coincident with this halting, irregular action of the heart, and it remained high until the normal rhythm returned. We found likewise that, in a slow or inhibited heart-action from exciting the vagus nerve with faradic current, the venous pressure rose. Hence it seems probable, as Plumier (61) states, that the rise in venous pressure after large doses of epinephrin is explained by the halting heart-action rather than by any venomotor stimulation" (p. 390). Bainbridge and Trevan (2) exclude reflex vagus inhibition in their experiments by a small dose of atropin early in each experiment. Under these conditions they find little or no change in vena cava pressure after adrenalin injection into a portal tributary or systemic vein (hepatic artery tied or intact), but a rise in portal pressure due, they think, either to a swelling of the columns of the liver cells narrowing the capillary channels, or constriction of the radicles of the portal vein. Two cubic centimeters of a 1 to 10,000 solution of adrenalin cause a rise in portal pressure equal to 255 mm. of sodium citrate solution. Kuno (50) explains the slight rise in venous pressure which he obtained on injection of adrenalin, with the heart beating faster, by saying, "the contraction of the blood vessels evoked by adrenaline is most distinct in the arterial system so that a large amount of blood might escape into the veins. The pressure in the veins does not therefore fall, on the contrary it rises more or less during action of the adrenaline although the heart works extremely energetically" (p. 232).

As far as I know, no measurements have been made of the effect of adrenalin on venous pressure in man. A word of caution should, therefore, be given about transferring data concerning adrenalin from animals to man. As has been noted above, the rise of venous pressure in dogs has been attributed to the concomitant slowing of the heart. In man, however, adrenalin does not slow, but quickens the heart rate.

Clinical findings reported by Donaldson (21) and Miller (54) indicate that in practically every person (normal or pathological) adrenalin injection causes no change or causes an increase in pulse rate. Only one case of a fall in pulse rate was reported (Donaldson) and this was in a patient much collapsed from hemorrhage. With the subcutaneous injecion of 0.5 cc. of 1 to 1000 adrenalin, used in the "Goetsch" test (29), (57), there is no reaction on the part of normal patients and an increase of ten to fifty beats per minute in patients hypersensitive to adrenalin. This result is further corroborated by the work of Wearn and Sturgis (74) and of Tompkins, Sturgis and Wearn (73). Therefore it would be incorrect to assume that the results obtained by Capps and Matthews (16) on dogs, in their studies on "venous blood-pressure as influenced by the drugs employed in cardiovascular therapy," necessarily apply to man. Similarly, Meek and Eyster's conclusion (53) (based on experiments on unanesthetized dogs with good vagal tone), that adrenalin cannot be the immediate cause of cardiac acceleration which follows moderate exercise, because the heart slows after adrenalin, cannot be held good for man in whom the heart increases its per-minute rate after adrenalin. Still another example of the different way in which adrenalin affects the same structure in different animals is given by Barbour (5), who found that adrenalin caused constriction of human coronary arteries, but relaxation of the coronary arteries of the calf, sheep and pig.

2. Various other influences. Since the experimental work in this paper deals only with the effect of adrenalin on venous blood pressure, it does not seem appropriate to review, in detail, the effect of various other influences on venous pressure. The following references may, however, serve to make this general review of the subject more complete. The relation of venous pressure to:

a. Gravity. Hill (35); Hill and Barnard (37); Barach and Marks (4)

b. Respiration. Jacobson (46); Wertheimer (75); Hill and Barnard (37); Burton-Opitz (12); Plumier (61).

c. Exercise. Burton-Opitz (11); Hooker (38); Elpers (23); Jones (47); Henderson and Harvey (33). Krogh's (49) recent work on the effect of exercise in opening up new capillary beds, is of interest in this connection.

d. High altitudes. Schneider and co-workers (66), (67), (68), (69); Kellaway's (48) work on the relation of anoxemia to the output of adrenalin may throw some light on the question of the effect of high altitudes on venous pressure.

e. Increased atmospheric pressure. Hill (36).

f. Drugs, other than adrenalin. Hill and Barnard (37); Plumier (61); Burton-Opitz (14); Capps and Matthews (16).

g. Injection of physiological solution. Bainbridge (1); Kuno (50).

Venous pressure work on man. A review of various methods for measuring venous pressure in man is found in an article by Hooker and Eyster (43), in the Johns Hopkins Hospital Bulletin for 1908. The following is a list of articles dealing with these methods:—Oliver, 1898, (58); Frey, 1899, (26); 1902, (27); Gaertner, 1903, (28); von Basch, 1904, (6); Sewall, 1905, (70); von Recklinghausen, 1906, (62); Oliver, 1907, (59); Moritz and von Tabora, 1910, (56); Frank and Reh, 1912, (25); A. A. Howell, 1912, (45); Lombard, 1912 (51); Hooker and Reese, 1914, (44); Hooker, 1914, (39); Brown, 1918, (10); Wiggers, 1918, (76). For comparison with the normal venous pressure values in man, given in the above articles, it may be of interest to note the venous pressure values obtained by Jacobson, 1867, (46), in sheep, and by Burton-Opitz, 1903, (12), in dogs.

Pathological values are given in the articles by Calvert (15); Hooker and Eyster (43); A. A. Howell (45) and Clark (17). Diurnal variations are given by Oliver (60); Hooker (39) and Clark (17); the effect of age and sex on venous pressure by Elpers (23) and Hooker (39), (40), and the effect of temperature by Elpers (23); Hewlett (34) and Hooker (39).

AN EXPERIMENTAL STUDY OF THE EFFECT OF ADRENALIN ON VENOUS BLOOD PRESSURE

Introduction. It is evident from the foregoing discussion that in studying the effect of any drug on venous blood pressure, one must take into consideration the effect of this drug on the various mechanisms, changes in which are known to cause changes in venous pressure. So close is the relationship between the various parts of the circulatory, vasomotor and respiratory systems, that any change in one part usually causes changes in several other parts. Hence, a change in venous pressure must very often be ascribed to the operation of several factors, sometimes supplementing, sometimes antagonizing one another. Sometimes one factor is so predominant than any other is lost sight of. This seems to have been the case in the previous study of the effect of adrenalin on venous pressure. A greatly slowed heart causes a rise in venous pressure. Adrenalin, injected into the blood stream of dogs with good vagal tone, causes slowing of the heart and a rise in venous pressure. Naturally, the conclusion was that the rise in venous pressure was due to the slowing of the heart rate, especially since little change in pressure occurred on administration of adrenalin to these dogs after vagotomy.

It has been demonstrated for sheep by Gunn and Chavasse (30), and for dogs and roosters by Crawford and Twombly (18), that adrenalin chloride causes contraction of isolated vein rings. The work of Bainbridge and Trevan (2) indicates that the radicles of the portal vein in the intact animal contract under the influence of adrenalin. Nerves to the veins of the limb have been demonstrated by Thompson (72) and Bancroft (3), and to the portal vein by Mall (52) and others. The experiments of Hooker (41), (42), demonstrate the existence, in the intestinal veins at least, of a nervous constrictor mechanism, which can be stimulated directly or indirectly. In view of this evidence, one naturally wonders whether or not such a nervous mechanism is functioning when adrenalin chloride is introduced into the circulation, and if the general venous pressure rise occasioned thereby is not, in part at least, due to a constriction of the veins. No evidence, as far as I know, has been found that points to such a mechanism coming into play after adrenalin, except in the case of the portal vein.

It is with these things in mind that the present investigation has been undertaken, to ascertain what factor or factors are responsible for the general rise of venous blood pressure which follows the intravenous injection of a solution of adrenalin chloride.

Experimental procedures. In the course of these experiments about fifty dogs and twenty-five cats were employed, weighing, on the average, 8 kgm. and 2.3 kgm., respectively. The experiments on dogs were carried out under ether anesthesia. The cats were decerebrated according to the method described by Sherrington in his recent laboratory manual (71), an anesthetic, ether, being used only during the procedures prior to the decerebration. The decerebrate cat preparations were always kept on an electric pad throughout the experiment.

Arterial pressure was recorded by a mercury or a Hürthle manometer, sometimes by both. Venous pressure was recorded as follows: brass cannulae (7 cm. long by 1 to 1.5 mm. bore, for cats, and 16 cm. by 2 to 2.5 mm., for dogs), connected with manometers containing 2 per cent sodium citrate, were inserted in the external jugular and femoral veins and pushed in past the valves so that the pressures recorded were those in the superior and inferior cavae. Whenever there was any doubt that the cannulae were not properly inserted, a dissection was made at the end of the experiment. The entrance of the superior cava into the heart was taken as the zero level for pressure. To make sure, from time to time, that no clots had formed, the pressure was raised in the manometer, by means of a pressure bottle, to several centimeters above the pressure existing in the vein and the citrate solution then allowed to run into the vein. This it did promptly, if there was no obstruction. It was always noted on the tracing when such a procedure occurred. Controls were made which showed that injections of such amounts of 2 per cent sodium citrate (½ to 1 cc.) had no visible effect on the circulatory system, and the amount of fluid injected was insufficient to cause any change in venous pressure.

Simultaneous tracings of arterial pressure, respiration and time relations were made on a smoked paper kymograph. Since the experimenter worked alone, it was necessary to resort to some method of recording, so that venous pressure readings could be made and injections carried out and recorded by one person, and the exact correspondence between the various pressures be noted on the tracing. One lever recorded respiration, another the arterial blood pressure, and a signal magnet, which marked the base line for the arterial pressure (mercury manometer), recorded the duration of the injection and was also connected with a Harvard clock recording half-minutes. In this circuit there was an electric buzzer which signaled every time the marker was recording a half-minute, and at the sound the level of the venous pressure manometers was noted, the pressures later being marked at the proper place on the tracing. On the same line with this marker, and writing only a millimeter or two behind it, a Jacquet chronograph recorded seconds.

Parke, Davis & Company's adrenalin chloride 1 to 1000 solution was used throughout. Before injection, it was diluted with 0.7 per cent sodium chloride to the desired strength, usually 1 to 10,000.

Special experimental procedures, not followed in all experiments, are described in connection with the experimental data obtained from those procedures.

Of course, if small rises of venous pressure are to be considered significant, one must make sure that they are not caused by the introduction of fluid, used in the injection. For this reason, very small amounts of adrenalin solution were slowly injected (in most cases 0.5 to 3.0 cc. in 5 seconds or more), and control experiments were several times made to ascertain how large an amount of 0.7 per cent NaCl had to be introduced to cause any change in venous pressure. One-half to 3.0 cc. were without effect, 5.0 cc. caused a rise of 3 or 4 mm., while 20.0 cc.—a larger amount than was ever employed in adrenalin chloride injections—caused a rise of only 10 or 15 mm. Na citrate solution.

Experimental work on: 1. Changes in peripheral resistance and capacity of the vascular system. Under all conditions studied, adrenalin always caused a rise in arterial pressure due to arterial constriction. The rise was higher for the same dose when the heart rate was increased than when it was decreased, but there was always a rise. Such a constriction, other things being equal, would tend to cause a fall in venous pressure due to the obstruction to the flow from arteries to veins but, on the other hand, the decreased capacity of the system brought about by arterial constriction would tend to raise the pressure in all parts of the circulatory system. In some cases of arterial constriction, as shown by Bayliss and Starling (7) and discussed in the historical review in this paper, the two factors may balance and give no change in venous pressure. There is one factor here which seems to have received little notice in this connection, and that is the rate at which plasma may leave the blood stream under varying arterial pressures. How far this may operate toward compensating the "decreased capacity of the system" factor, is not known. At all events, in the case of adrenalin, when all other causes for change in venous pressure are ruled out, as far as possible, these factors of peripheral resistance and capacity of the system seem to balance, for no change in venous pressure occurs. Chart 1 (I and II) illustrates this point. About a quarter of an hour after the decerebrated cat (chart 1, I) was curarized, according to the procedure described under "respiration," 0.15 mgm. (3 cc.) of adrenalin chloride was injected into the right femoral vein causing no change in vena cava pressure, although the arterial pressure was raised from 34 to 167 mm. Hg.

Chart 1 (III and IV) shows an experiment in which the venous pressure fell after adrenalin, a very unusual occurrence. It is probable that, in this case, the increased peripheral resistance so obstructed the flow from arteries to veins that there occurred a fall in venous pressure. The decrease in heart rate would tend to cause a rise in venous pressure, but this was apparently overbalanced by the factor or factors tending to cause a fall. In any case, it seems evident that the usual rise in venous pressure caused by adrenalin is not brought about, directly, by changes taking place in the arteries, though, as will be explained later, a rise in arterial pressure may assist in causing a rise of venous pressure when it occurs in conjunction with a slowing of the heart.

2. Chemical regulation. It is conceivable that adrenalin may act as a chemical stimulant to venous musculature, independent of any effect

CHART NO. 1

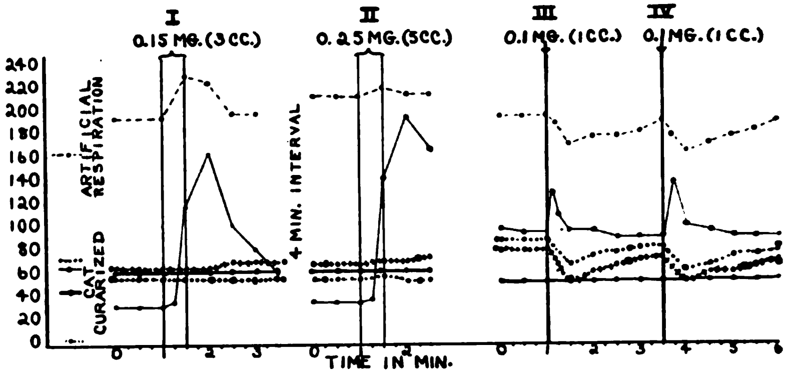


CHART NO. 2

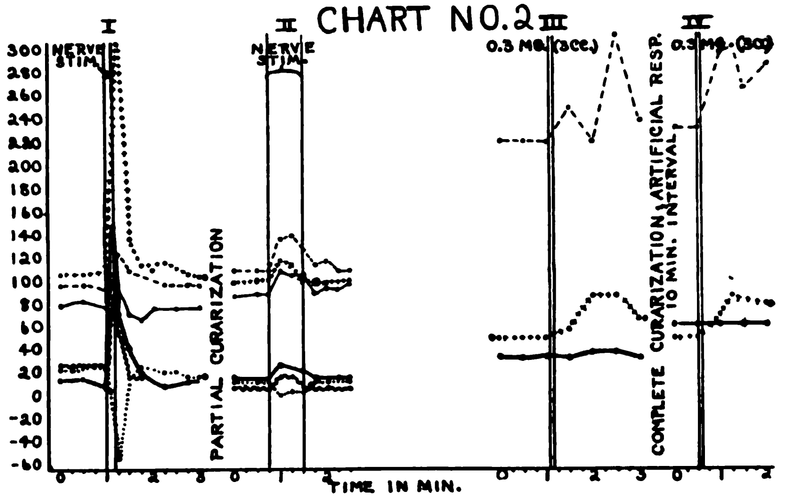
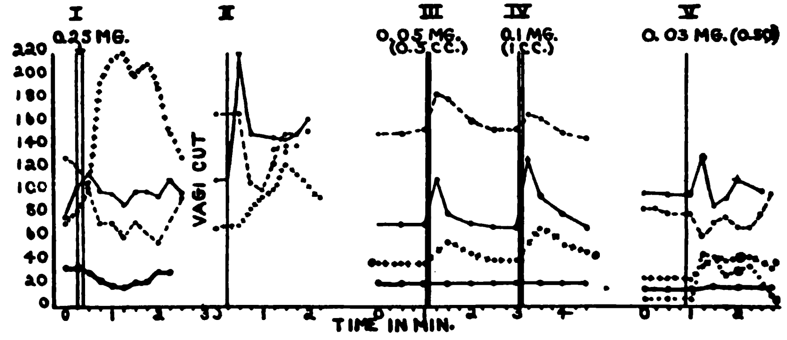


CHART NO. 3



through a nervous mechanism. As far as I know, no work has been done on this subject. However, adrenalin produces an effect upon venous rings similar to that upon arterial rings, and it seems reasonable to assume, until disproved, that in the veins, as has been shown for the arteries, adrenalin acts by stimulating the myoneural junctions of the sympathetic nerve fibers in the vascular musculature.

3. *Respiratory and muscular factors.* The contraction of muscles against the veins, as in exercise, and changes in respiration, are known to be very efficient in causing changes in venous pressure. Take for example chart 2 (II), showing the result of stimulation of the saphenous nerve before and after administration of curare. The dog was not completely curarized, as can be seen from the fact that a slight change in rate and amplitude of respiration was elicited by strong sensory stimulation. The interesting thing is that the venous pressure response seems to bear a definite relation to the amount of respiratory response. When adrenalin chloride is injected in small amounts (usually 0.1 to 0.3 mgm.) there may be no respiratory response, or a decrease in height and frequency of respiration occurs. This change evidently has little or nothing to do with the rise in venous pressure, since that rise takes place when no respiratory variations occur, and a decrease in rate causes a fall, not a rise in venous pressure. However, to fully rule out any change in venous pressure due to muscular contraction or respiratory change, curare was administered in a number of experiments, and as soon as breathing ceased artificial respiration was given. The air was heated to 30°C. before reaching the animal. To be sure that other muscles besides the respiratory muscles were paralyzed, the

------ pulse rate per minute.
——— arterial pressure in mm. Hg.
.... superior cava pressure in mm. 2 per cent sodium citrate.
++++ inferior cava pressure in mm. 2 per cent sodium citrate.
━━━ respiratory rate per minute.
Wavy line, respiratory amplitude (relative only).
Large dots indicate actual determinations.
Circles indicate that venous pressure cannula was tested and found free of clots.

Chart 1. Adrenalin chloride injections: I and II, decerebrate cat 7; III and IV, dog 4 B.

Chart 2. I and II, dog 35 A—Stimulation of saphenous nerve before and after partial curarization; III and IV, decerebrate cat 9—Adrenalin chloride injections, before and after complete curarization; vagi cut.

Chart 3. Adrenalin chloride injections: I and II, dog 1 A, vagi cut between I and II; III and IV, dog 4 B; V, dog 29 A.

minimal nerve stimulus necessary to cause contraction of some skeletal muscle in the fore limb was determined before curare, and then sufficient curare was given to cause this stimulus and other stronger stimuli to become ineffective. In such experiments adrenalin had practically the same effect on the venous pressure before and after curare: chart 2 (III and IV) is an example. Changes in the rate of the artificial respiration seem, also, to be ineffective. It has been noticed a number of times that the venous pressure response to adrenalin is not as marked immediately after the administration of curare as it is later. In a few cases, as shown in chart 1 (I and II), the effect persists. This depressant action on venous pressure response is probably due to an action on the nervous mechanism in the veins. That it usually passes off very rapidly, fits in with the general idea that the primary depressant action of curare on the circulatory system is of very short duration.

When not curarized, occasionally the act of injecting was a sufficient sensory stimulus to cause a muscular response. This muscular response, often seen in a stretching out of the lower limbs and consequent stretching of the abdominal muscles, was not evident on the tracing given by the usual pneumograph. Therefore, instead of recording respiration by means of a rubber bag around the chest, a small rubber balloon was inserted through a small opening into the abdominal cavity near the diaphragm, and the skin closed tightly around the glass tube to which the balloon was attached. The balloon was then blown up until it held about 5 cc. air, and connected with a recording tambour and lever as the bag around the chest had been before. This has the advantage of recording not only respiratory changes but also changes in abdominal pressure which very markedly influence venous pressure, as shown by Hill and Barnard (37).

4. Heart rate and output per minute. Plumier (61), in his work on venous pressure, laid great stress on slowing of the heart rate as a very efficient means of raising the venous pressure. Occasionally the heart suddenly stops beating after an injection of adrenalin. The venous pressure may then rise very high; in one case it rose five times as high as it had after a similar dose of adrenalin when the heart rate was increased. In dogs with good vagal tone, the heart usually slows markedly after adrenalin and the venous pressure rises (see chart 3, I). Even with the vagi cut the pulse rate is often slowed, due probably to a direct action on the heart musculature, as is shown in chart 3 (II). A rather uncertain venous pressure response is often seen in vagotomized dogs when the heart rate is increased after an adrenalin injection.

Such results would lead one to attribute the rise in venous pressure solely to the decreased heart rate as did Plumier (61) and, later, Capps and Matthews (16). Chart 3 (III, IV and V), however, makes one doubt that heart rate is the only factor involved. Work on dogs under ether did not prove satisfactory in clearly demonstrating whether or not a nervous mechanism played some part in venous pressure response to adrenalin. There are several reasons for this. In order to rule out the slowed heart rate, the vagi had to be cut. This caused a very great increase in the depth of respiration which brought about such a marked rise and fall in venous pressure that it was difficult to compare such fluctuations in pressure with the change occurring after adrenalin, since the respiratory variations were then often absent, due to temporary cessation of respiration. Besides, even with the vagi cut, the heart frequently slowed after adrenalin. Still more serious, perhaps, is the possible effect of ether on the nervous mechanism. Ether depresses the arterial vasomotor response to adrenalin, as shown by Berry (8) and Rous and Wilson (63), and could naturally be expected to affect a nervous mechanism in the veins—all the more so because, as shown by Hooker (41), this mechanism is extremely sensitive. If very light ether anesthesia were employed, there would then be the difficulty of muscular and respiratory responses discussed above. Chart 3 (III and IV) shows an unusual experiment in which the ether anesthesia was light, and yet respiration remained constant throughout the experiment. Experiments on decerebrated cats, which needed no anesthetic after the operation, and whose vagal tone is not nearly so marked as dogs', proved to be very satisfactory. Also, it was no small consideration, since curare is very difficult to obtain, that it took only a small amount of curare to curarize a cat, as compared with a dog. Since sensory stimuli have been shown to be effective after curare, it is desirable, for humanitarian reasons, to work on a decerebrated preparation where there can be no question of not giving enough anesthetic during curarization to cause analgesia.

Concerning the influence of change of the heart rate on venous pressure, it should be said that it is really the per-minute output of the heart, rather than the heart rate alone, that is essential. Quoting from Erlanger and Hooker (24), (p. 161), "If the pulse pressure is approximately dependent upon the amount of blood that escapes from the arteries during one cardiac cycle, then it is obvious that the amount that escapes must vary directly as the pulse rate." Therefore, pulse pressure multiplied by pulse rate gives us an expression of the output of the heart per minute. It is possible to increase or decrease the output per minute by increasing or decreasing either or both of these factors, and to keep the output constant by varying the two factors proportionately in the opposite directions. Hence one cannot say that a slowed heart necessarily causes a decreased output per minute, and hence a rise in venous pressure. It is theoretically possible that the pulse pressure might so increase that even with a lower heart rate the output per minute would increase. In the case of adrenalin, however, this possibility seems never to be realized, at least when the pulse rate is greatly reduced. For example, in one experiment, before adrenalin, the pulse pressure was 180 mm. Hg. and the pulse rate per minute 158, making a per-minute output of 28,440. After adrenalin, the pulse pressure was 240 and the pulse rate 20, giving the greatly decreased per minute output of 4800. The slowing in this case was way out of proportion to the increased pulse pressure, and was accompanied by a rise in venous pressure from 25 to 120 mm. Na citrate.

Experiments were performed on dogs, as illustrated in chart 4, in which the vagi were cooled by perfusing ice water through glass tubing in a V around each nerve, and afterwards allowing the nerves to come to room temperature again. The pulse rate, arterial pressure, respiratory rate and amplitude were all affected but very little change in venous pressure took place. When the perfusion of ice water around the nerves was discontinued, the pulse rate decreased from 144 to 108, the venous pressure decreasing also a few millimeters. When the heart stops beating the rise in venous pressure is due to a back pressure in the veins and also to the tendency for equalization of pressures to take place throughout the vascular system. When the heart slows, but does not stop, these factors operate to cause a rise in venous pressure, but to a less extent. If now the arterial pressure rises as the slowing of the heart occurs, as after adrenalin, the mean pressure of the vascular system toward which the various pressures are tending will, of course, be greater, and hence there is a much greater possibility of a large rise in venous pressure. In one experiment, after an adrenalin injection, the heart was slowed about as much as after the cooling of the vagi was discontinued (chart 4, II). In the former case the arterial pressure rose, showing that the capacity of the vascular system was decreased, while in the latter it fell. In the case of adrenalin there may be a nervous constrictor mechanism in the veins at work, but from other experiments on dogs it seems likely that this plays little part in etherized dogs. It seems more probable that the decreased capacity of the system acts in connection with the slowed heart rate to cause a rise in venous pressure.

5. Nervous mechanism. It can readily be seen from the foregoing that one cannot attribute a rise in venous pressure to the activity of a vasomotor mechanism in the veins, unless one is sure that the per-minute output of the heart has not decreased sufficiently to explain the rise. As we have seen, when this factor is ruled out in experiments on dogs under ether, the evidence for the functioning of a nervous mechanism is not very conclusive. In the experiments on decerebrate cats, however, there is clear evidence of such a mechanism. With the vagi intact, we may get the response which is usual in dogs—a rise in venous and arterial pressure and a slowing of the heart, chart 5 (I and II). When, however, the vagi are cut (chart 5, III and IV), there is still a rise in venous pressure. The heart rate is practically unchanged and the pulse pressure increased (as nearly always occurs after adrenalin) so that the output per minute is now increased and the tendency would be for a fall rather than a rise in venous pressure. So also in this experiment, the respirations were decreased in height and frequency which would tend to lower rather than raise venous pressure. The rise, therefore, which one does get under these circumstances seems to be due to a nervous factor, rendered probably less effective in producing a rise because of the factors tending to produce a fall. Chart 6 shows very uniform rises in venous pressure, though in some cases the heart rate is slowed and in some cases increased in frequency. In the experiment shown in chart 7, the respiratory factor is completely controlled by curarization. The vagi are cut in IV and V.

It is interesting to inquire whether or not the seat of this action of adrenalin is central or peripheral. Two different types of experiments were performed to investigate this question. In the first, the cord was sectioned in the cervical region (about at the level of the fifth or sixth cervical vertebra). As is shown in chart 8, this does not destroy the venopressor response to adrenalin, though whether or not any small part of the reaction is of central origin is hard to determine, since the amount of response to a certain dose is not invariable, and so the effect of a dose before cutting the cord is difficult to compare with the same dose just afterwards. The response before and after is quite similar, however.

For the second type of experiment the isolated vein preparation of Hooker (41) was used. In the first of these experiments, Doctor Hooker kindly demonstrated the method to the writer. An isolated loop

CHART NO.4

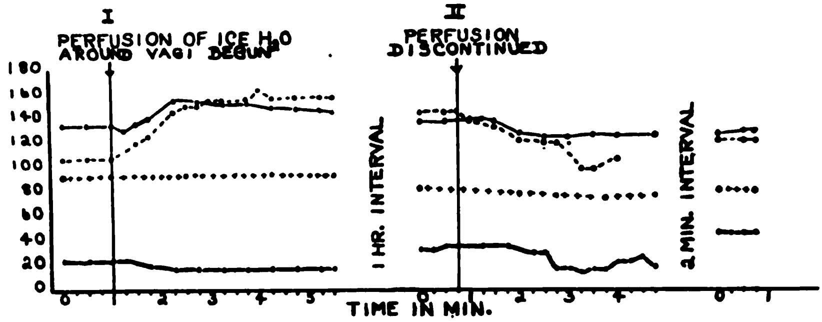


CHART NO.5

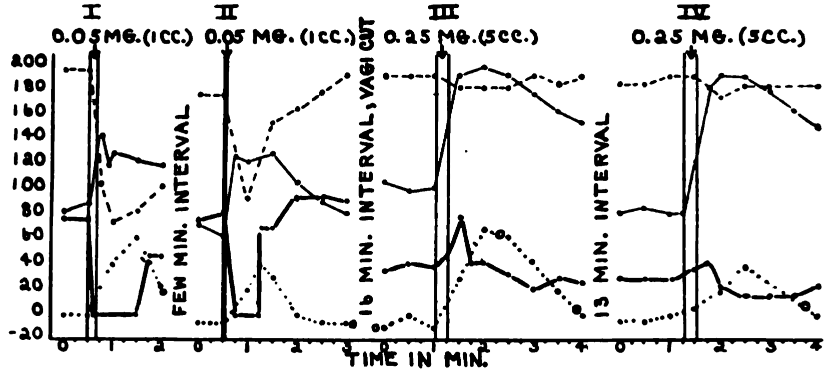
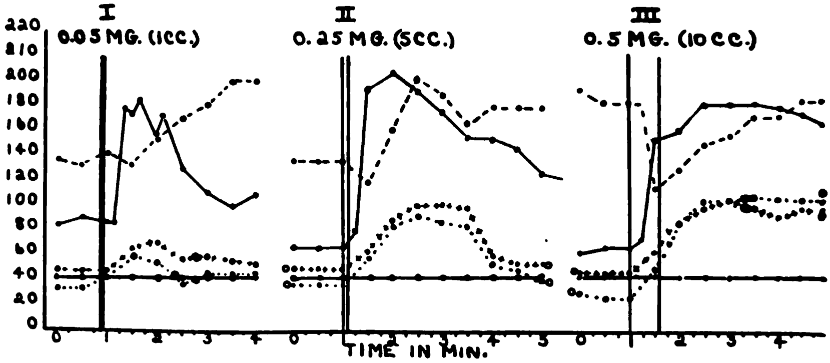


CHART NO.6



of large intestine in which the inferior mesentery artery and vein had been cannulated, was hung up free from the surrounding intestines. The only connection with the animal was through a nerve running from the inferior mesenteric ganglion to the large intestine and entering the intestine in close proximity to the inferior mesenteric artery. Blood was washed out of the vessels from artery to vein by means of warmed (about 37°C.) Ringer's solution under air pressure of 90 to 120 mm. Hg. The cannulated artery was then disconnected from the air pressure apparatus and allowed to hang freely from the preparation. The vein was then connected with a venous pressure manometer containing warmed Ringer's solution. The zero level was, in most cases, adjusted to the level of the vein in which the pressure was being measured. The vein was distended by a positive pressure of from 30 to 100 mm. Ringer's solution, by means of a pressure bottle. Any change in the caliber of the vein would then be indicated by a rise or fall in the level in the venous pressure manometer. Hooker (42) has shown that, in this preparation, a rise in venous pressure may be elicited, peripherally, by stimulation of the nerve to the part; reflexly, by sensory stimulation; and centrally, by asphyxia. In such a dog, when adrenalin chloride was injected into the saphenous vein in doses from 0.2 to 0.6 mgm., no rise in venous pressure in the isolated vein occurred, but when such injections were followed by asphyxia, produced by closing off the end of the tracheal cannula for two or six minutes, a rise in venous pressure from 15 to 20 mm. occurred, showing that the preparation was still in good condition. This seems to show that the venopressor rise after adrenalin is not due to any central mechanism, controlling the caliber of veins, but to a peripheral effect on sympathetic endings, such as takes place in the arterioles.

------ pulse rate per minute.
——— arterial pressure in mm. Hg.
.... superior cava pressure in mm. 2 per cent sodium citrate.
++++ inferior cava pressure in mm. 2 per cent sodium citrate.
━━━ respiratory rate per minute.

Large dots indicate actual determinations.

Circles indicate that venous pressure cannula was tested and found free from clots.

Chart 4. Dog 17 A—Vagus nerves cooled.

Chart 5. Decerebrate cat 3—Adrenalin chloride injections, vagi cut between II and III.

Chart 6. Decerebrate cat 6—Adrenalin chloride injections, artificial respiration.

CHART NO. 7

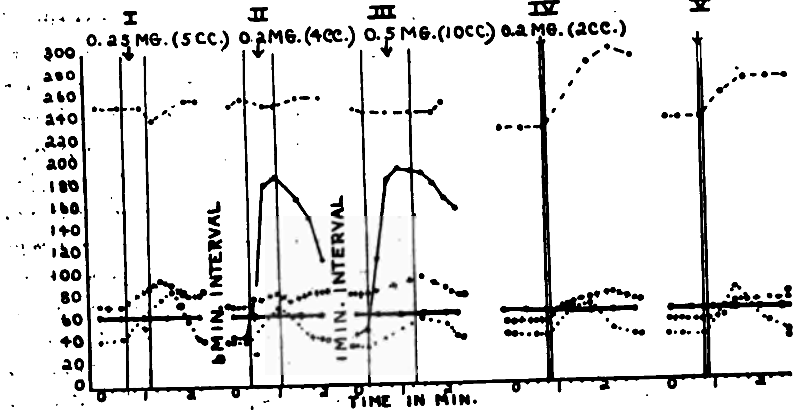


CHART NO. 8

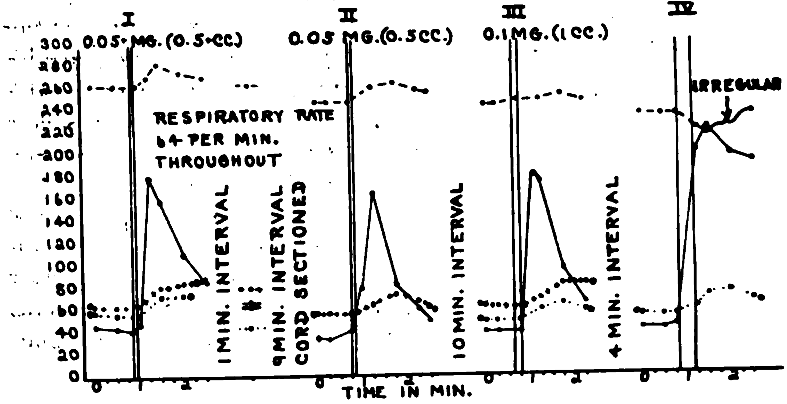
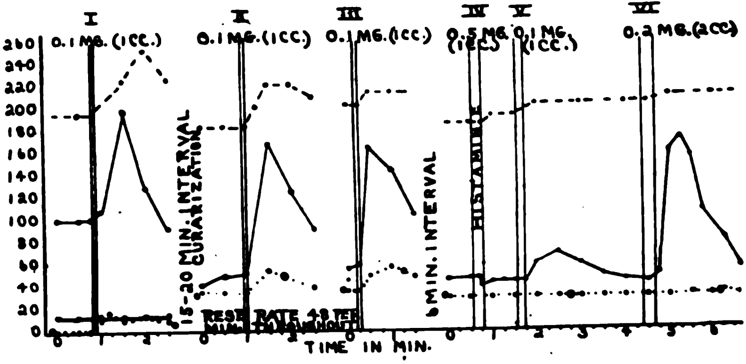


CHART NO. 9



Some experiments which were being carried out in this laboratory suggested that histamine might have an effect on the venopressor mechanism. Chart 9 (I, II, III) shows typical arterial and venous responses to adrenalin in a curarized, decerebrate cat. One-half milligram (1 cc.) of histamine (chart 9, IV) caused a fall in arterial pressure; in some experiments the venous pressure also fell. A more marked reaction was shown in another experiment, where twice the dose was given. According to Dale and Laidlaw (19), had the cat been under an anesthetic, these doses of histamine, from which the unanesthetized cat recovers, would have produced fatal circulatory collapse. When histamine was followed by adrenalin in the same and larger doses than given previously in the experiment, there was no venous pressure response (chart 9, V and VI). The arterial pressure rose, but not nearly as high as before, with the same dose. A second injection, however, gave a typical arterial pressure rise, but still no change in venous pressure. The work of Dale and Richards (20) and Dale and Laidlaw (19) leads them to conclude that relaxation of the capillaries is the cause of the vasodilator effect of histamine. The fall in venous pressure after histamine and the subsequent failure of the nervous mechanism to respond to adrenalin, indicates that histamine has a relaxing effect on venous tone, also, and acts in some way to depress the activity of the venopressor mechanism.

Summary. The various possible factors operating to produce the rise in venous blood pressure which occurs in dogs and cats after intravenous injection of adrenalin are discussed. The two factors chiefly responsible are the decreased heart rate bringing about decreased unit output of the heart, and a vasoconstrictor mechanism in the veins. The

- - - - - - pulse rate per minute.
———— arterial pressure in mm. Hg.
.... superior cava pressure in mm. 2 per cent sodium citrate.
++++ inferior cava pressure in mm. 2 per cent sodium citrate.
▬▬▬ respiratory rate per minute.
Large dots indicate actual determinations.
Circles indicate that venous pressure cannula was tested and found free from clots.

Chart 7. Adrenalin chloride injections: I, II and III, decerebrate cat 8—Vagi intact; cat curarized; artificial respiration; IV and V, decerebrate cat 9—Vagi cut; cat curarized; artificial respiration.

Chart 8. Decerebrate cat 15—Adrenalin chloride injections, before and after section of cord; vagi cut; cat curarized; artificial respiration.

Chart 9. Decerebrate cat 17—Adrenalin chloride injections, before and after histamine; vagi cut; cat curarized and artificial respiration begun between I and II.

effect of the first factor is accentuated by the fact that the arterial pressure is greatly raised by adrenalin in the doses here used. The first factor has been recognized before. Reasons are given why the second factor was previously overlooked. In dogs with good vagal tone and under an anesthetic, the rise in venous pressure is almost entirely due to the first factor. In cats whose vagal tone is not nearly as strong, the second factor predominates. This nervous mechanism is shown to be acted on peripherally by adrenalin, and to be depressed by ether, curare and histamine, especially the last.

I may take the opportunity here to thank Dr. W. H. Howell and Dr. D. R. Hooker of this University, and Dr. J. L. King of Goucher College, for kindly encouragement and aid in the preparation of this paper.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

II. LUNG AUTOMATISM AND LUNG REFLEXES IN THE SALAMANDERS (NECTURUS, AXOLOTL)

A. B. LUCKHARDT AND A. J. CARLSON

From the Hull Physiological Laboratory of the University of Chicago

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In a previous article (5) we showed that the lung of the frog contracts immediately following the external respiratory act; that the lung musculature can be induced to contract reflexly by the stimulation of any visceral or cutaneous nerve with practically no exceptions; that the lungs of this animal possess a peripheral automatic mechanism which at times shows rhythm but which under normal conditions is kept in a state of inhibition by tonic central inhibitory impulses via the vagi; that these nerves, furthermore, contain not a few motor fibers which apparently exert no marked tonic activity; and that the cervical sympathetic nerves contain but few motor and no inhibitory fibers for the lungs. In addition, we made a brief study of the action of certain drugs (atropine, adrenalin and histamine) on the lungs besides using nicotine extensively in differentiating between the motor and inhibitory nerves contained in the vagus fibers to the lungs.

On completion of this work it seemed quite important to us to investigate the neuro-muscular apparatus and physiology of the nerves of the lungs of other available amphibia to determine whether or not the physiological mechanism discovered in frogs is the same or similar in this class of animals.

METHODS

The only amphibia available for this study were the axolotl and necturus. The general methods of study were those reported in our first article, modified to meet the anatomical peculiarities of the type of animal which we were studying.

In every instance the cord was pithed below the medulla. As in previous work on the frog we cannulated the tips of the lungs exposed by a ventral incision and connected each cannula with either a water manometer or delicate tambour. After recording the normal respirations or respiratory attempts of the animal, we attempted to elicit reflex lung contractions as the result of mechanical and electrical stimulations applied to the animal anterior to the spinal transection. We subsequently closed the glottis with a small mosquito forceps (in axolotl only) and pithed the brain (specifically the medulla) to note any tonic inhibitory influence the vagi nerves might have on the neuromusculature apparatus of the lungs. Stimulation of the same nerves to the lungs next engaged our attention. This was followed by the effect of the intravenous injection of drugs alone or in combination. Because of the poor or imperfect circulation in necturus, as a result of the preparation of the animal for experimentation, for certain purposes we injected the drug deep muscularly, 15 to 20 minutes, before preparing the animal in the manner described above; for we found that even injection of the drug in Ringer's solution by way of the bulbus arteriosus or into the pulmonary artery failed to reach the posterior end of the lung of this animal.

The lungs of necturus are paired sacs, extending from the level of the heart almost to the anal region. There are no alveoli or septa in these lungs. According to Miller (9) the course of the smooth muscle fibers in the lung walls is circular, except at the apex. Both lung artery and lung vein lie superficial to the muscle layer.

Miller has described medullated and non-medullated nerve fibers and nerve nets in the necturus lung. The pulmonary fibers of the vagi enter the base of the lungs in many branches, not along the pulmonary blood vessels, as in the frog, but between the blood vessels. There are many ganglion cells (bipolar and multipolar) in the main nerve trunks and in the nerve plexuses. According to Miller, the non-medullated fibers connect with the ganglion cells. Similar ganglionated nerve plexuses have been described in the lungs of other tailed amphibians, especially by Stirling (11) in the case of the newt. The older anatomical literature is reviewed in detail by Oppel (10). Miller's description of the ganglionated nerve plexuses in the necturus lung is practically identical with the local nervous system of the necturus heart, as studied by one of us (6).

In necturus the glottis or opening from the pharynx into the trachea is exceedingly small, and certainly not suited for rapid filling or emptying the lung sacs with air. We have repeatedly seen these animals attempt to swallow air, the air escaping by the gill slits and in no instance entering the lungs. There can be no doubt that in necturus the gaseous exchange is carried out by the gills, supplemented by the skin, and the rôle of the lung sacs in respiration is an open question. The lung sac of necturus has the same histogenesis as the lung of other vertebrates, although it remains most primitive as regards differentiation. Should these structures be called lungs, especially if they serve mainly or exclusively as "hydrostatic organs?"

In our review of the anatomical and zoölogical literature on amphibian respiration, we came across the surprising fact, apparently well known to zoölogists though not to physiologists, that many species of tailed amphibians have neither lungs nor gills, and in other species without gills the lungs appear to be too rudimentary to function in respiration. The normal condition of these species is that of the frog with the lungs extirpated, that is, the gaseous exchange is carried out entirely by the skin. But several zoölogists (Wilder (12), Lönnberg (8), Camerano (4), Bethge (3) and others) have concluded mainly on anatomical grounds (great vascularity of the mucous membrane of the buccal cavity and upper end of the esophagus), that the pharyngeal cavity serves lung functions, especially in those species having neither lungs nor gills. It appears to be a fact that the so-called buccal respiratory movements (including that of the external nares) are carried out even in those species in which the lungs are absent. But Babák and Kühnova (1) have shown that the brain centers controlling these movements are different from those governing the filling and emptying of the lungs, the latter are, the former are not influenced by asphyxia, which fact seems to throw doubt on the respiratory character of these buccal movements. Lapique and Petetin (7) have also shown, in the case of one species of salamanders devoid of lungs and gills, that the main respiratory organ is in the skin.

None of these questions can be settled except by direct physiological experiments, but we may regard the necturus lungs, provisionally at least, as lungs on the basis of organogenesis. Their motor control also places them in the same category with the lung of the frog and of the axolotl.

Since the entire respiratory apparatus (including the lung) of the necturus is strikingly more primitive than that of the other salamander used in this study, we shall describe the results obtained from necturus first and end with a description of those obtained from the axolotl.

Necturus maculatus. External respiration in this animal is essentially performed by the large gills which under usual conditions are kept in more or less constant motion. Occasionally the animals come to the surface for air which is promptly expelled by an act of swallowing through the gill slits. The lungs of this animal consist essentially of two thin elongated muscular sacs which are well supplied with blood vessels. With but one exception we found them collapsed. In an inflated and atonic condition their diameter is about 1 cm. at their greatest circumference. At their ends they taper off into blunt tips, at their base they communicate with a tracheal sac similar to that possessed by the frog. This sac communicates with the oral cavity through a glottis situated far down in the pharyngeal region. The glottis is exceedingly primitive. It consists essentially of a slit which is quite easily overlooked on direct inspection. We agree with the description of Oppel that the glottis is exceedingly delicate. Taking everything into account we feel that the lungs may serve an excretory function (elimination of CO_2) but are rarely if ever filled with air during any act of external respiration. The lungs receive their innervation through pulmonary fibers carried by the vagi. It was found impracticable to isolate these latter nerves in the neck for direct electrical stimulation. They were isolated at their exit from the skull by a dissection to right and left of the median line after a sagittal section of the skull.

Because of the inaccessibility of the glottis for closure by hemostat as practised in the frog, we prevented communication of the lungs through the glottis with the mouth by dorso-lateral traction and fixation in that position of the fore legs after pinning the animal on its back. A wad of cotton wedged under the neck of the animal at the level of the tracheal sac or put over the tracheal sac and held firmly in that position by the constriction of a rubber band was an additional measure employed in not only closing off the glottis but in preventing intercommunication between the lungs through the tracheal sac.

Axolotl. The lungs of this genus of amphibia are certainly more complex than in necturus. On opening the abdominal cavity one is at first glance struck with the resemblance of the lungs of this animal with the reptilian lung. The upper portions of both lungs are subdivided into alveolar sacs by septa; the lower appendages which extend down the abdominal cavity for a considerable distance are more saclike in their texture. As in the frog and necturus the lungs communicate at their base with the tracheal sac which in turn communicates with the mouth cavity through a well-developed glottis. In the specimens which we used gills were present and functioning. It was equally apparent, especially on opening the abdominal cavity, that the animals could and did make use of the lungs for gaseous exchange. In this animal the vagi could be isolated in the neck as in the frog. The result of our investigation on this form is confined to a study of less than a dozen animals. Although most of the animals were in good condition at the time of experimentation, they deteriorated more quickly than the frog and the necturus similarly prepared. We are confident, however, that our results are characteristic of this form especially since they fit in well with what we observed in the frog and necturus.

All the tracings reproduced with this report were taken with the same speed of the kymograph. A single time tracing showing 5 second intervals will be found at the bottom of figure 1. It can be used in a study of the time relations in all other tracings should the reader care to do so.

RESULTS

1. Necturus maculatus: a. The central inhibitory control of the lungs through the vagi. As mentioned above it was found impossible for anatomical reasons to isolate the vagus nerve or its pulmonary branches in the neck to note the effect of ligation and sections of these nerves on the tonic activity of the lung musculature. Since destruction of the medullary centers in the frog effected the same result, we adopted this expedient in necturus. Having closed the glottis and prevented intercommunication of the lungs as described above we connected the lungs each with a water manometer and destroyed the brain entirely by rapidly pithing it. Figure 1, *A*, shows the effect of such a procedure. The destruction at *a* of the cerebral lobes and midbrain was followed by a temporary escape of the lungs from inhibitory control. The subsequent destruction at *b* of the medullary centers was followed by permanent hypertonic state of the lung as in the frog. In another animal whose cord was not pithed at all but which lay quietly on its back as a result of a rubber band placed tightly about the front legs,[1] ligation of the base of one lung was followed by an immediate escape of this lung from tonic inhibitory control in a manner identical with destruction of the medulla (fig. 1, *B*).

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[1] Both in the frog and in the necturus pressing the front legs tightly together with a rubber band renders the animals quiescent, and they will lie quietly on their backs for long periods without attempting to turn to a normal position. This procedure depresses also some of the skeletal reflexes.

It should be noted that in this animal the entire central nervous system was intact and that there was the minimum trauma produced in exposing the tip and base of one lung. Nevertheless, on severing this lung from its physiological connection with the central nervous system, the typical lung tetanus was produced. The results of this type of experiment strengthen our position that the striking peripheral automatism of the amphibian lung is a normal physiological state and not induced by the trauma rendered necessary by the experimental procedures.

From these experiments it would seem to follow that in this animal the lung is kept in tonic inhibition by central vagal control. If now one proceeds further and stimulates the peripheral end of the exposed vagus nerves at the base of the skull as was done in many animals, one obtains temporary escape of the lung from the hypertonic state as is shown in figure 2. This animal suffered at *a* an exposure of the anterior end of the brain by resection of the end of the upper mandible. This operative procedure was followed possibly by a slight inhibition of the lung tonus. The medulla was rapidly pithed at *b* resulting in permanent hypertonus of the lung. Stimulation of the peripheral end of the left and the right vagus at *c* and *d* respectively was in each instance followed by a temporary inhibition of the lung with an unusually rapid return to its hypertonic state. If the peripheral vagus stimulation is of sufficient strength the inhibition of the lung tonus usually puts the lung back temporarily to the identical tonus state prior to the destruction of the medulla. This is additional evidence that the state of the lung tonus in the intact animal is governed by the inhibitory control through the vagi.

Since ligation of the base of the lung effects the same result as destruction of the medulla, namely, an escape of the lung from its state of inhibitory control, it might be expected that electrical stimulation of the base of the lung would cause an inhibition comparable if not identical with stimulation of the peripheral end of the vagus. Figure 3 records the result of such an experiment. The lungs being in a state of hypertonus as a result of destruction of the medulla by pithing, stimulation of the vagus of the right lung at *a* caused an inhibition which is virtually duplicated by stimulation of the base of the left lung at *b*.

It appears to us from these observations that there is no escape from the conclusion that the vagi nerves possess inhibitory fibers for the lung which under normal conditions exercise a maximum inhibitory control over the lungs by tonic impulses from the medullary centers.

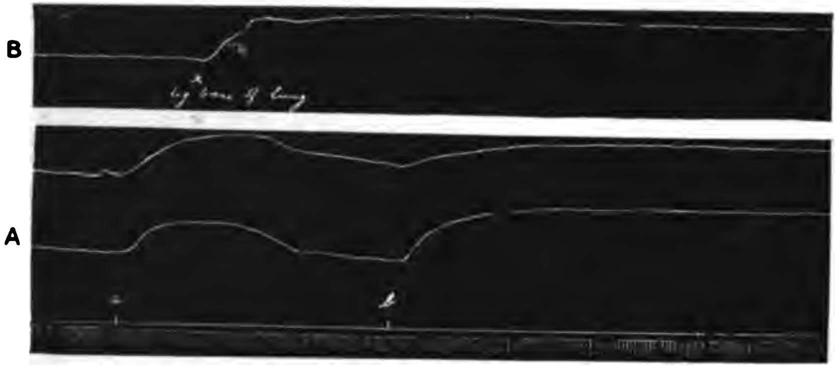

Fig. 1

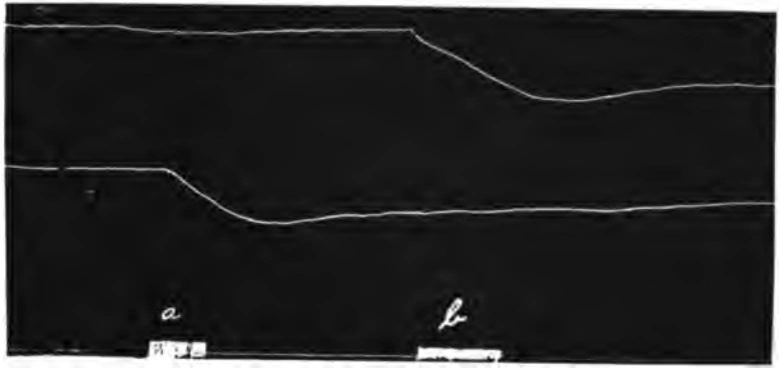

Fig. 3

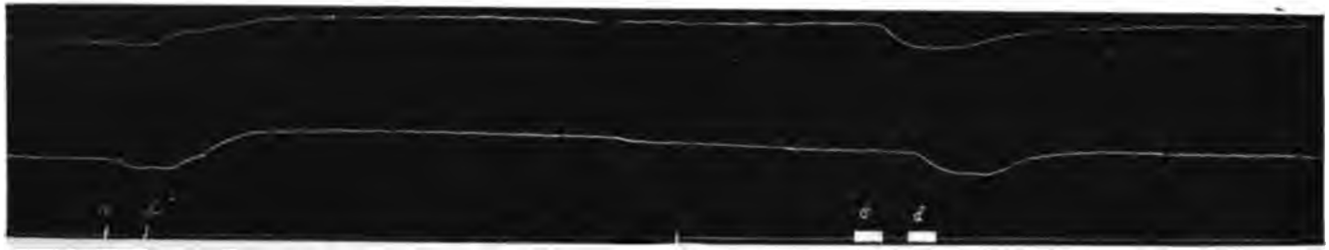

Fig. 2

b. Peripheral lung rhythm. In two instances the lungs of necturus showed a type of tonus rhythm seen occasionally in the frog. Figure 4, *A* and *B*, are records taken from these animals. In figure 4, *A*, this tonus rhythm appeared on pithing the medulla at *a*. Here a fast and later a slow tonus rhythm is written on the curve indicating release of the lung from central vagus inhibition. In figure 4, *B*, the tonus rhythm followed a decidedly rapid inhibition resulting from faradization of the base of the lung at *a* with a strong tetanizing current.

The interpretation of these results is based entirely upon speculation. It is possible that the rhythm which appeared as a result of the destruction of the brain (fig. 4, *A*) arose from occasional inhibitory impulses reaching the lungs through the vagi from more or less intact portions of the medulla which escaped complete destruction during the pithing of the latter. The tetanization with a strong current of the base of the lung in figure 4, *B*, at *a* might have effected changes in the physiological state of the automatic tissue present in the lungs which initiated an occasional and recurring refractory state of this peripheral automatic

Fig. 1. Water manometer tracings of the intrapulmonic pressure in necturus.

A: Spinal cord cut and destroyed below medulla, cannula in tip of lungs. Glottis closed by dorsal traction on front legs. Lungs isolated by ventral median incision; *a*, destruction of cerebral lobes and midbrain; *b*, destruction of the medulla. Showing permanent lung hypertonus on destruction of medulla.

B: Animal rendered quiet by tying rubber band around front legs, placed on dorsal side, no restraint, with water running over gills. Abdominal incision at base and tip of lung; *x*, ligation of base of lung. Showing permanent lung hypertonus identical with that following destruction of medulla. Time tracing: 5 second intervals.

Fig. 2. Water manometer tracings of the intrapulmonic pressure in necturus. Upper tracing, left lung; lower, right lung. Spinal cord cut and destroyed below medulla; cannulae in tips of lungs. Lungs isolated by a ventral median incision. Glottis (trachea) closed by pressure exerted by dorsal traction on front legs.

a, Transverse section of upper mandible exposing anterior end of brain.

b, Pithing brain.

c, Stimulation of left vagus at base of skull.

d, Ditto, right vagus.

Showing hypertonus of lungs induced by destruction of the medulla, and inhibition of this tonus by vago stimulation.

Fig. 3. Water manometer tracings of the intrapulmonic pressure in necturus. Cannulae in tips of lungs. Spinal cord and brain destroyed. Lungs in hypertonus. Upper tracing, left lung; lower, right lung; *a*, stimulation of right vagus at base of skull; *b*, stimulation of base of left lung.

Showing identical inhibitions of the lung tonus by vagus and by direct lung (base) stimulation.

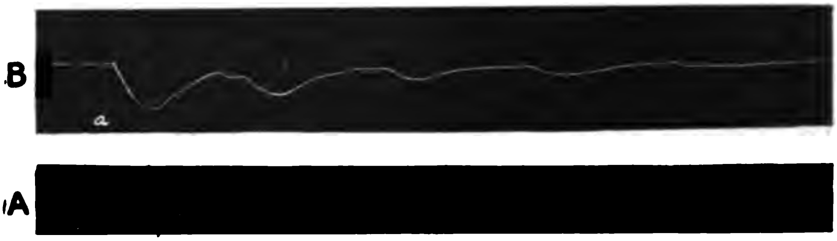

Fig. 4. Water manometer tracings of the intrapulmonic pressure in necturus. Cannula in tip of lung. Spinal cord cut and destroyed below medulla, lungs isolated by ventral median incision.

A: A pithing of the medulla showing a tonus rhythm of the lungs following destruction of the brain.

B: Lung in hypertonus from destruction of the brain; *a*, direct stimulation of the base of the lung with a weak tetanizing current.

Showing primary inhibition of lung tonus followed by a tonus rhythm.

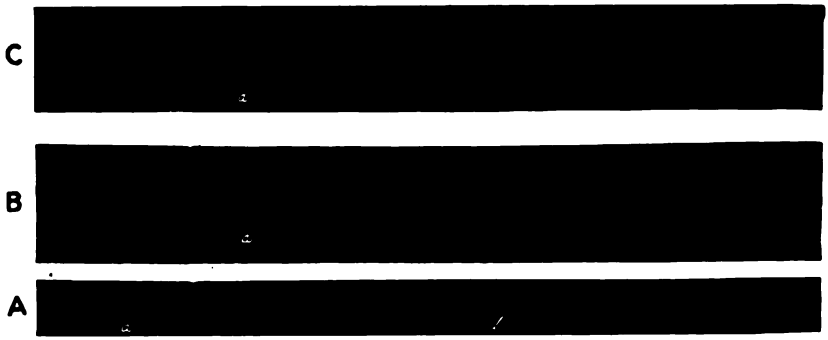

Fig. 5. Water manometer tracings of the intrapulmonic pressure in necturus. Cannula in tip of lung. Brain and spinal cord destroyed, giving the lungs permanent hypertonus.

A: *a*, injection 0.5 cc. adrenalin; *b*, 0.6 cc. of (1:1000) adrenalin in 5 cc. Ringer's into heart. Showing inhibition of lung tonus by adrenalin.

B: *a*, injection of 3 mgm. nicotine into heart, showing inhibition of lung tonus by nicotine.

C: *a*, injection of 0.6 cc. histamine (1–1000) into heart after partial recovery of preparation from a previous injection of nicotine. Showing direct stimulating action of histamine on lungs after paralysis of inhibitory nerve mechanism by nicotine.

mechanism, the inhibition of the hypertonic state of the lung giving rise to the appearance of a rhythm.

c. Reflex lung contractions as a result of cutaneous stimulation. We have been unable to effect reflex contractions of the lungs of the necturus by electrical or mechanical stimulation of cutaneous nerves anterior to the spinal transection.

d. The action of pituitrin, histamine, nicotine and adrenalin on the hypertonus of lung. In most of the work on drugs a cannula was tied into bulbus arteriosus. The drug was diluted with Ringer's and the injections made under moderate pressure through the bulbus. The method was poor even under favorable conditions. Direct intravenous injection was out of the question because active circulation through the lungs was absent in every animal although heart appeared in good condition. Even when the drug was injected under pressure we never felt certain that it reached all parts of the lung. This was due partly to the peculiarity of the pulmonary circulation in this animal and partly to the fact that a good deal of the fluid containing the drug escaped from the vessels ruptured in destroying the brain and isolating the vagi.

Adrenalin. The injection of adrenalin caused a marked inhibition of the hypertonic lung as is recorded in figure 5, tracing *A*, at *a* and *b*.

Pituitrin. According to the commonly accepted view, pituitrin is a direct stimulant of smooth muscle tissue. We were somewhat surprised to find that this drug in small or large doses causes a prompt and marked inhibition. Figure 6, *A*, is a record taken from an animal which suffered destruction of the medulla at *a* followed by a hypertonic state which was markedly reduced by the injection of pituitrin at *b*. The slow recovery is also recorded. No attempt was made to determine the point of action of pituitrin. Pituitrin not only caused a relaxation of the lung; but when weak solutions of this drug were used to irrigate the intestines all intestinal movements ceased after a short latent period. It would seem, therefore, that in these animals pituitrin does not act as a direct muscular stimulant but rather as a stimulant of the inhibitory mechanism.

Histamine. Figure 6, *B*, illustrates at *c* the typical inhibitory effect of this drug in an animal whose lungs were in hypertonus as a result of destruction of the medulla at *b*. Both lungs showed pronounced relaxation with very slow recovery. In all types of animals other than necturus this drug causes a more or less powerful contraction of the lung musculature (turtle, frog). Necturus proved to be an exception

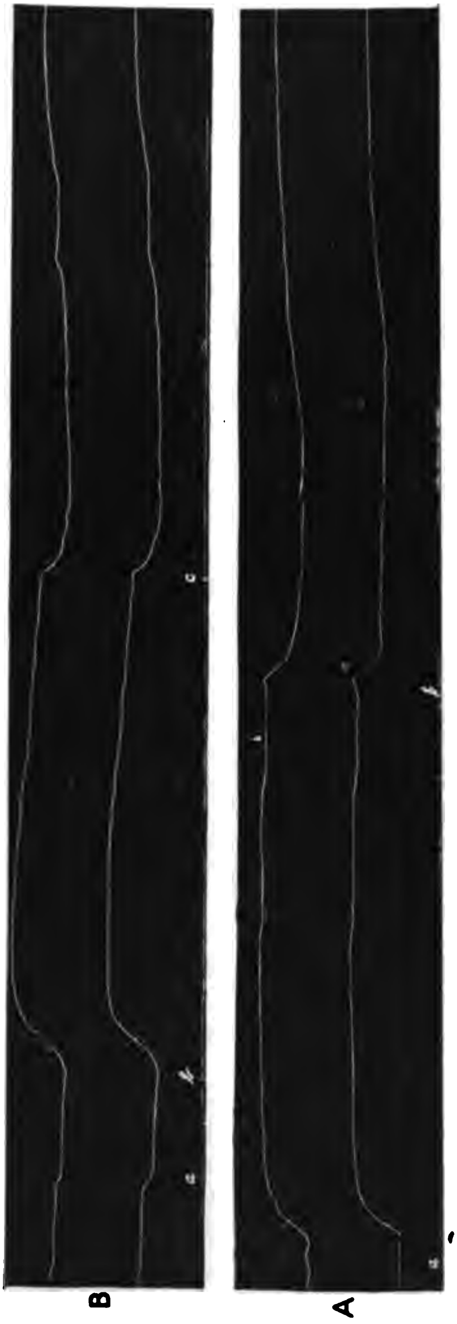

Fig. 6

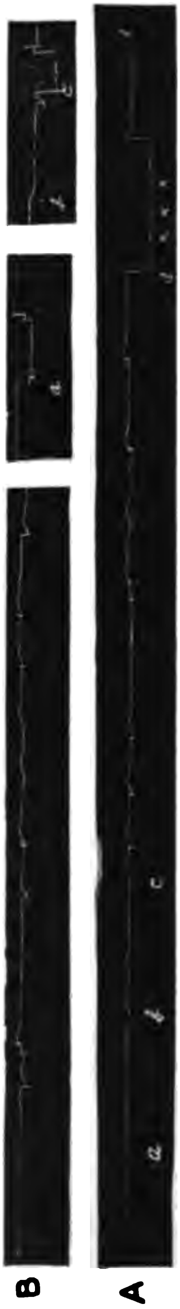

Fig. 7

to the generally accepted view that the drug supposedly acts as a direct muscular stimulant. The results obtained suggest the possibility that the drug acts primarily on the inhibitory mechanism. We therefore attempted to eliminate the latter by means of nicotine. As a matter of fact we found that the injection of histamine after previous nicotinization effected a contraction of the lung in every instance as is recorded graphically in figure 5, *C* at *a*.

Nicotine. It will be recalled that this drug acted on the frog's lung in a manner similar to section of both vagi. We furthermore showed that in this animal the drug acted by paralyzing the inhibitory center in the medulla as well as the vagus inhibitory terminations in the lungs; for we found that after nicotinization stimulation of the peripheral end of the vagus gives rise to a lung contraction instead of the inhibition seen before giving this drug.

The subcutaneous or deep muscular injections of large (5 to 10 mgm.) doses into the normal intact necturus is in 15 minutes followed by general clonic convulsions with an increase in the rate and amplitude of the gill movements. Tetanus of the gills supervenes and active external respiration comes to an end some time before the general

Fig. 6. Water manometer tracings of the intrapulmonic pressure in necturus. Cannula in tip of each lung. Spinal cord cut and destroyed below medulla. Lungs isolated by ventral median incision. Glottis and tracheal sac closed by mechanical pressure through dorsal traction on front legs.

A: Upper record, left lung; lower, right lung; *a*, pithing of brain; *b*, injection of 0.5 cc. pituitrin in 5 cc. of Ringer's solution into heart. Showing inhibition of lung hypertonus by pituitrin.

B: Upper tracing, left lung; lower, right lung; *a*, section of upper mandible exposing anterior end of brain; *b*, pithing of brain; *c*, injection of 0.7 cc. histamine (1–1000) in 5 cc. Ringer's solution into heart. Showing inhibition of lung hypertonus by histamine.

Fig. 7. Axolotl. Water manometer tracings of the intrapulmonic pressure. Spinal cord pithed and destroyed below level of innervation of front legs. Lungs isolated by abdominal incision; cannula in tip of lungs. Glottis open. Spontaneous respiration (quick movements of lever) except where indicated.

A: *a*, Gentle mechanical stimulation of gills.
b, Gentle mechanical stimulation of skin of front legs.
c, Gentle mechanical stimulation of skin of mandibles.
d, Strong mechanical stimulation (pressure) of toes of front leg.
x, strong attempt at respiration (swallowing).
B: *a*, Moderate mechanical stimulation of the gills.
b, Gentle stroking of skin of front leg.
c, Strong mechanical stimulation of the gills.

Showing lung contractions following spontaneous respiratory movements and on stimulation of various sensory nerves.

convulsions cease. The lungs of such animals were found contracted. *Pithing the brain, stimulation of the vagi themselves, or stimulation of the lung itself at its base were without effect.*

If the dose of nicotine injected subcutaneously is reduced still further (2.5 to 1 mgm.) the same symptoms appear in 10 to 15 minutes but are less severe. Pithing of the brain or stimulation of the vagi is again without effect. Direct stimulation of the lung at its base with a strong tetanizing current gives rise to an inhibition of the lung without any or with but feeble return.

From these results it would appear that *a*, the lungs of this amphibian is supplied only with inhibitory fibers through the vagi; *b*, that nicotine paralyzes the respiratory center for the lungs and also the junction between the preganglionic fiber endings and postganglionic cell body, since external respiration ceases with the lungs in hypertonus and since stimulation of the base of the lungs will still yield inhibition when stimulation of the vagus nerve itself gives nothing; *c*, the vagus nerve contains no motor fibers for the pulmonary musculature. Without commenting at this time on the possible significance of this fact, we call attention to the fact that in this most primitive lung which we have studied, the lung is solely under the tonic influence of inhibitory fibers carried by the vagi and motor fibers are apparently absent. We have been unable to elicit a motor response of the lung either by stimulation of the vagi or the lung itself in any normal or nicotinized animal.

The apparent resistance of the peripheral lung mechanism to this drug compared with that of frog may possibly be due to the rather sluggish and imperfect circulation through the lungs.

2. Axolotl. Earlier in this paper we called attention to the fact that the axolotls with which we worked, although possessing gill remnants, were essentially air-breathing animals; and that the lungs were decidedly better developed for that purpose than the lungs of the necturus.

a. Lung contractions at the end of normal respiration. Records were taken of the changes in the intrapulmonic pressure occurring during normal respiration with the glottis open. It can be seen from figure 7, *B*, that every spontaneous respiratory effort (gulp) as indicated in the tracing of the quick movement of the lever is followed by contraction of the lung. These lung contractions compare favorably with those obtained under similar experimental conditions from the frog.

b. Reflex lung contractions. Gentle mechanical stimulation applied to the skin of the mandible, gills or front legs induced lung contractions of reflex origin as is seen in figure 7, *A*, at *a*, *b* and *c*. In these three instances the lung contraction appeared without a preceding attempt at respiration. Subsequent attempts at respiration were followed in each instance by lung contractions in every way similar to those of reflex origin induced by gentle stimulation. It is obvious that the contractions are not the result of changes in the intrapulmonic pressure due to movements of the animal during external respiration; for they occur in the absence of all visible movement of the head region.

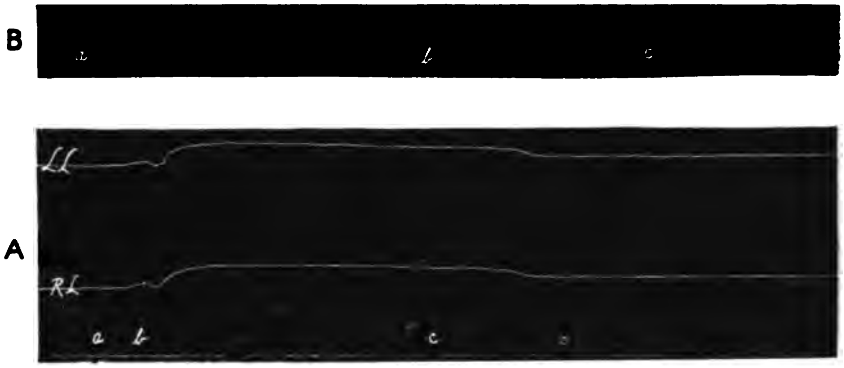

Fig. 8. Axolotl. Water manometer tracings of the intrapulmonic pressure. Spinal cord transected below medulla and pithed posteriorly. Cannula in tip of lungs, lungs isolated by median abdominal incision. Glottis closed by forceps. All injections intravenously (in 2 cc. Ringer solution).

A: Lower tracing, right lung; upper, left lung.
a, Transection of upper mandible exposing anterior end of brain.
b, Pithed brain.
c, Injection of 1 cc. 1:10,000 adrenalin.
B: a, Pithed brain.
b, Injection of 0.01 cc. 1:1000 histamine.
c, Injection of 2 mgm. nicotine.

Showing the inhibitory action of these drugs on the lung hypertonus following destruction of the brain, the latter being equivalent to section of the vagi nerves.

Strong mechanical stimulation of the fore legs or gills may lead to partial or complete opening of the glottis with partial or complete collapse of the lungs as is illustrated in figure 7, *A*, at *d*, and figure 7, *B*, at *a*, *b* and *c*. Strong attempts at respiration after marked stimulation of pain fibers as at *xxx* in figure 7, *A*, were not followed by the filling of the lungs until considerably later. It might be assumed that the strong stimulation interfered temporarily with the central control of the mechanism normally at the service of the animal in filling its lungs with air or depresses the central inhibitory control over the lungs. If the latter is the correct interpretation the animal failed to fill its lungs in spite of violent respiratory attempts because of their hypertonic state. Although we have no direct evidence on this matter the latter interpretation seems to be the more probable one.

c. The central inhibitory control of the lungs through the vagi. It is certain that in the axolotl the medullary centers exert, under normal conditions, a tonic inhibitory control over the lungs as in the frog and necturus; for as a result of the destruction of the medulla which is equivalent to section of both vagi (as in fig. 8, *A*, at *b*) both lungs go into a state of hypertonus.

d. Action of certain drugs on the hypertonic lungs. Adrenalin. This drug temporarily inhibits the hypertonus of the lungs resulting from destruction of the medulla as seen in figure 8, *A*, at *c*.

Histamine. In axolotl the intravenous injection of 1 mgm. of histamine-HCl caused inhibition of the lung musculature not unlike the action of this drug in the hypertonic lung of necturus (fig. 8, *B*, at *b*).

Nicotine. The invariable effect of nicotine on the hypertonic lung of the axolotl is inhibition (fig. 8, *B*, at *c*).

As previously noted we were not only restricted to a few animals in study of this form but found that the physiological state of the animals rapidly declined as the result of our operative procedures. The axolotl furnishes a less hardy physiological preparation than the frog or necturus. As far as our results go they check with those obtained from the frog and necturus.

SUMMARY

1. The vagus center exerts a tonic inhibitory control over the lungs. Destruction of the medulla releases the lung from this control. As a result, the lungs assume a state of more or less permanent hypertonus (necturus and axolotl).

2. Electrical stimulation of the peripheral end of the vagus nerves causes a temporary inhibition of the hypertonic state of the lungs, only on the side of stimulation, during and for some time after the stimulation (necturus and axolotl).

3. The efferent vagi fibers to the lungs are solely of the inhibitory type. We have never seen the slightest indication of a motor response on stimulation of these nerves. Unlike the frog, the vagi of these forms of amphibian life possess few if any motor fibers for the lungs (necturus and axolotl).

4. Electrical stimulation of the base of the lung yields the same results as stimulation of the peripheral end of the vagus (necturus and axolotl).

5. We have been able to elicit reflex lung contractions from gentle mechanical stimulation of cutaneous nerves only in the axolotl. Intense stimulation of sensory nerves (pain) probably prevents filling of the lungs during attempts at respiration because of a hypertonic condition of the lungs due to an inhibition of the inhibitory center.

6. There may appear as a result of destruction of the medulla or strong faradization of the base of the lung a tonus rhythm in the denervated lungs of the necturus. This rhythm has not been seen in axolotl.

7. Adrenalin, pituitrin, histamine and nicotine cause a marked inhibition of the hypertonic condition of the lungs resulting from the destruction at the medulla. Histamine injected into the animal after nicotine causes a contraction of the lung. In axolotl these drugs act in the same direction. Pituitrin was not used in this form; nor was histamine injected after nicotinization of the animal.

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CHANGES IN ACID AND ALKALI TOLERANCE WITH AGE IN PLANARIANS

WITH A NOTE ON CATALASE CONTENT

JOHN W. MACARTHUR

From the Hull Zoölogical Laboratory, University of Chicago, and the Department of Biology, University of Toronto

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During a study of the effects of certain typical acids, bases and salts upon living planarians (*Planaria dorotocephala*, *P. maculata* and *P. velata*), observations were made which are here reported on account of the wider interest of their bearing upon tolerance of H^+ and OH^- ions, upon the relative efficiency of the mechanism for regulation of neutrality in young and old individuals, and upon the problem of acidosis in general.

Methods. A closely graded series of concentration of the acids (hydrochloric chiefly, also sulfuric and acetic) or alkali (sodium hydroxide) is made up from standardized N or 0.1 N solutions by dilution with aerated well water or other (Lake Michigan or Lake Ontario) water in which the worms live and thrive. The well water, which was used chiefly, has a pH value of 7.5 to 7.6 and an ion and gas content to be published with the larger study above mentioned. Both distilled water and strongly chlorinated tap-water are in themselves injurious to these worms and hence could not well be used for the purposes of these experiments; but similar tests are now being made with *P. maculata* in distilled water, this species being but little affected by distilled water in the period of time required.

Into the series of dilutions of an acid or alkali in 500 cc. or 1000 cc. Erlenmeyer flasks, filled and ready to be plugged with rubber stoppers, are introduced the flatworms, usually ten larger (18 to 20 mm.) and ten smaller (8 to 12 mm.) specimens together, all selected sound from established well-fed cultures. In some cases a similar group of three easily distinguishable sizes was used (22± mm., 15± mm. and 8± mm.). In control flasks all such individuals live practically indefinitely.

The hydrogen ion concentrations, already in a graded series from the method of diluting the normal solution, are measured and corrected at critical points by the colorimetric method with appropriate indicators: thymol-blue, brom-phenol-blue, methyl-red, brom-cresol-purple, phenol-red and phenol-phthalein, and the Hynson, Westcott and Dunning apparatus (1). Certain difficulties were encountered from the fact that glassware requires particularly thorough cleaning after each usage, and because strong acids added to water containing so much carbonate as do these naturally generate CO$_2$ and such solutions tend to return gradually toward neutrality. But these facts have no great significance here except as they render impracticable a precisely accurate determination of the actual limits of tolerance, as may be made in distilled water and by electrometry. In all cases it was the relative rather than the absolute susceptibility that was sought and that is here emphasized.

Extensive physiological studies of planarians have been made by Child (2), (3), who showed for *P. dorotocephala* by various means that the smallest worms (up to about 6 or 7 mm.) consist of but one zoöid, while the medium-sized ones (12 to 14 mm.) usually possess a second zoöid region, and larger specimens (20 to 25 mm. or more) exhibit at the posterior end a third zoöid or group of very small zoöids, constituting what is essentially a "growing tip." At least the chief, the second and the third of these zoöids were demonstrable physiologically by both "direct" and "indirect" methods in cyanides, but only after fission do the typical head structures of the zoöids become visibly differentiated morphologically. The growing tip is evidently involved repeatedly in the reproductive process; hence as this region grows its zoöids become more independent and acquire a higher rate of metabolic reaction and, like young individuals, are more susceptible to high concentrations and less susceptible to low concentrations of lethal agents (KNC, anesthetics, etc.).

Experimental. The chief observations are recorded and summarized graphically in the accompanying figure. The time records are averages from repeated tests made of each dilution; such averages for separate tests show but small deviations from the general average, but individual deviations are often large and overlapping wherever the curves lie close together. Dr. C. M. Child, to whom the writer is indebted for many opportunities and suggestions in this work, has recently used these experiments as part of a class course at the University of Chicago.

Acids. Immersed in HCl solutions that kill almost instantly (e.g., in acidities greater than about pH = 2) all worms are fixed and preserved intact (range of preservation). In lower concentrations, from pH = 2 down to about pH = 4.5, all individuals are killed and caused to disintegrate, the older somewhat later and more slowly than the younger (range of direct susceptibility and inhibition). In this disintegration all regions of the body are not equally and simultaneously involved, but usually the posterior tip and the head are first attacked and then the regions behind the head in order from in front backward; and, as might be expected from the small differences in direct susceptibility between young and old, the anterior end of a second zoöid is not distinguished. As the acidity approaches pH = 4.5 or pH = 4.6, the age difference in survival time decreases more and more and finally

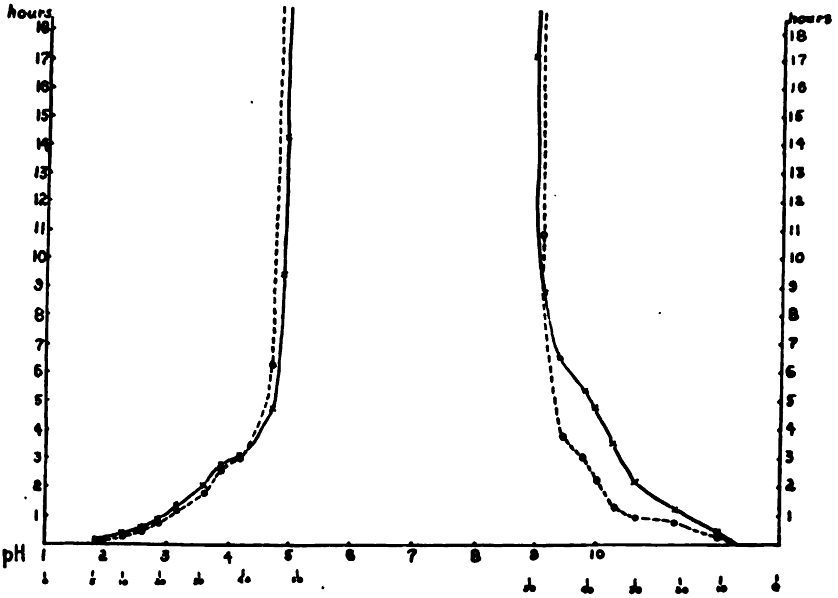

Fig. 1. Time in hours of distinct initial disintegration of young worms ---o---o--- and old worms ——x——x—— in solutions of different pH values (abscissae) up to pH = 10. Small numbers below abscissae indicate volumes of well water added to one of N/10 HCl (left) or NaOH (right).

Below pH = 2 ± is the range of preservation or fixation. Then follows with increasing dilution up to pH 4.4 ± the range of distinct direct susceptibility which grades off by a transition range into the range of acclimation or indirect susceptibility.

With NaOH the range of preservation is evidently absent, direct susceptibility differences between young and old much more marked, and the indirect susceptibility differences less marked than with acids.

becomes nil, and disintegration does not discriminate between the small and the large. In still more dilute solutions, however (pH = 4.7 to 4.9), where death is delayed for several hours and a certain amount of recovery from the initial inhibitory effects of the agent is possible, the relative susceptibility of young and old individuals is reversed (range of indirect susceptibility and acclimation), and smaller worms disintegrate last or not at all, while larger ones either disintegrate entirely or lose their head region. Posterior zoöids are left intact, and the posterior part of the first zoöid was never seen to disintegrate before the anterior end. Often the young worms live for days or indefinitely after the old are partially or wholly gone. Recovery tests, made by returning young and old alike to fresh water after various periods of exposure to the agent, are even more delicate in their indication of the sites and degrees of injury, and were used to extend and confirm the results obtained by leaving the animals in the agent up to the end of the experiment.

A more or less definite sequence of changes leads up to the final disintegration with acids. After the initial stimulation, during which the flatworm assumes for a time a slender form, moves rapidly and secretes some mucus, it gradually loses its power of adherence to the glass walls of the container and becomes shortened, cylindrical and swollen. This state is soon followed by discoloration or whitening, the loss of color occurring, as noted, first at the sensory tip, margins and ventral surface of the head and gradually extending backwards, often more rapidly on the ventral surface, and in larger individuals beginning early also at the growing tip. The whitening appears to indicate that semipermeability or some similar property of the surface layer has been abolished at the approach of death, for following close upon the loss of pigment occur disintegrative changes of a characteristic kind: as the parts become sticky and adherent to glass the regularity of the external contour is interrupted by small breaks in the continuity of the surface, the protoplasmic granules swell and mass into small clumps or liquid spheres and scatter out into the medium until finally little remains of the old body but a soft white shreddy outline composed of the more resistant connective and supporting tissues quite stripped of all the relatively susceptible epithelial parts.

Results differ in no essential way if sulfuric acid in slightly higher concentrations be substituted for the hydrochloric, or if a considerably greater strength of acetic acid be used—the difference probably being necessary to compensate for the lesser dissociation of the organic acid.

A *change of response* occurs upon addition of acid to the normal medium. The planarians then exhibit a fairly strong negative geotropism, climbing always up the walls of the container, whether in doing so they approach or attain a surface or not. Since strong acids cause a release of CO_2 into the solution, the response may perhaps be considered generally appropriate and adaptive, inasmuch as ordinarily an increase of CO_2 is doubtless associated with an insufficiency of oxygen (to which a similar response is made) and both could doubtless be avoided by rising to a better aerated surface layer.

Alkali. In alkaline solutions the same general results are obtained with certain more or less significant modifications. In the hydroxide (NaOH) stimulation is evidently more marked than in acids, and both whole worms and surviving parts of any size are more active both spontaneously and upon mechanical stimulation up to the very point of death. Alkalies also cause the secretion of a very excessive amount of mucus, which collects, as often as removed, in the bottom of the vessel.

Even quickly killing concentrations do not produce a definite fixation and preservation. Disintegration, if rapid, occurs, by a rather violent process of splitting and bursting of the dorsal surface in darkened lines; if slow, it begins at the margins and dorsal surfaces of the head and the posterior tip, and in larger specimens may also appear at what is presumably the anterior end of the second zoöid.

Results with NaOH differ from those with HCl and resemble more nearly those with KNC in one respect—in the slowly acting concentrations, allowing partial acclimation of the larger animals, death sometimes begins at the posterior end of the first zoöid and proceeds forward, while the head region of the first zoöid and all of the posterior zoöids remain intact for some time or indefinitely. In short the details of disintegration with this alkaline agent resemble those with most "acid dyes," while "basic vital dyes" rather resemble acids in their effect (unpublished work).

By the method of direct susceptibility there is much greater difference in survival time of young and old with NaOH than with HCl, the old surviving about twice as long as the young. The effective range of concentrations for indirect susceptibility, on the other hand, is less extended than with acids.

It will be noted that the range of critical concentrations, within which young animals and young parts only are able to regulate slight H^+ ion alterations, is a comparatively limited one and lies just beyond the limits resisted by all alike. Thus increase of H^+ ion up to pH =

4.9 on the one side or of OH^- ion to about pH = 9.1 on the other come within the normal range for all members of the species; slight additional changes (from pH = 4.9 to pH = 4.8 or 4.7 and from pH = 9.1 to pH = 9.2 or 9.3) can be met by the young individuals and parts alone; still greater changes are beyond the powers of acclimation of any, though the old resist the longer.

The greater tolerance by younger planarians and the posterior zoöid region of such dilute acid and alkaline solutions is almost certainly only another example of the greater power of acclimation to mildly depressing conditions associated so generally with more active metabolism (3). In fact the general principle underlying the indirect susceptibility method is founded on the discovery that organisms or parts of organisms possessing an intenser metabolism can acclimate or acquire tolerance more quickly and more completely than less active organisms or parts to low concentrations of cyanides, narcotics, etc. Child also showed later that the anterior, ventral and median regions (the regions of high direct susceptibility and presumably of most rapid metabolism) in Echinoderm and Annelid embryos, developing in low concentrations of NaOH, alcohol or HCl in sea-water, acclimated or acquired tolerance, or after temporary exposure recovered most quickly and underwent a proportionately accelerated and increased development in the larvae (4).

The explanation of this power of acclimation is not known, but may be in some way associated, as regards acids and alkalies, with differences in protoplasmic conditions, such as the higher percentage water content of metabolically active parts or individuals. If this water carries, as seems probable, at least an equal proportional and a greater total salt content, such inorganic salts of these as are buffer-acting substances (carbonates, phosphates, etc.) would act here much as in mammalian blood, to increase resistance to additional H^+ and OH^- ions in the medium. Or, if a greater proportion of mid-products of protein metabolism, or more ionized protein, be present during rapid metabolism, then these amphoteric substances may serve as acids or bases according as there are excess bases or acids in the medium. Naturally such buffers and metabolites would be protective only against slightly and slowly injurious concentrations; with higher concentrations the projective action is quickly overcome and the agent may diffuse and act most rapidly in the parts with greatest water content.

The acid or alkali effects may of course be produced through injury to some enzyme or enzymes essential to continuance of metabolic processes. Inasmuch as the almost universally occurring enzyme, *cata-*

lase, may eventually be shown to play some rôle in metabolism generally and in oxidation in particular (5), the writer wishes to record here the results obtained from numerous experiments to determine the catalase content or activity of planarians of different ages. It was found that equal weights of crushed young worms (8 to 15 mm.), maturer worms (18 to 20 mm.), and of very old worms (25 to 30 mm.) liberated in 15 minutes at 22°C. from 1½ per cent unneutralized hydrogen peroxide the following quantities of oxygen respectively per gram weight of tissue: 653.3 cc., 460.4 cc. and 317.6 cc. Without a single exception, in many repetitions of the experiment, the rule was found to hold that *the larger (older) the worm the lower is the catalase content.* It is of interest to note that the oxygen consumption of young planarians has been found to be from 15 per cent to 100 per cent greater than that of old ones (6), showing a higher basal metabolism, just as the Benedict method does for man.

Some significance should be attached to the fact that though there are such large differences in direct susceptibility of young and old with alkalies, these differences are small with acids; while, on the contrary, though the differences by indirect susceptibility are small with alkalies, they are larger with acids. *The young are evidently comparatively and absolutely less resistant to alkalies, but relatively more resistant to acids.* With advancing age there would appear to be a decreased relative resistance to acids and an increased relative resistance to alkalies—a set of changes such as would result from a gradual onset of a state of acidosis and the more or less incomplete oxidation of the larger-acid products left from a state of lowered metabolism. MacNider (7) has shown that as age advances the acid-base equilibrium of mammals is more and more easily disturbed or overtaxed; that uranium nitrate, for instance, is more toxic to the old than to the young and produces sooner in the old a condition of true acidosis (8), characterized by a depletion of reserve carbonates in the blood, etc.; and that the aged, after uranium treatment, received intravenously without injury considerably more alkali than did the young.

For all the species used by Child (4) in controlling form and proportions of developing embryos, he found that "the agents which are most effective in producing the differentially inhibited type of form are least effective in producing the types of form characteristic of differential acclimation, and vice versa," his series being effective in producing acclimation types in the order: HCl, alcohol, NaOH, NH_4OH, KNC. Thus acclimation was rapid in acids and alcohol, slow in NaOH and

NH_4OH, and exceedingly slow in KNC. A similar contrasting physiological effect between HCl and NaOH is here shown by other means for fresh-water planarians.

Comparing allied species with *P. dorotocephala* it may be said that in general *P. maculata* has a slightly wider range of normal tolerance, while *P. velata* is distinctly less resistant to acids and more resistant to alkalies.

SUMMARY AND CONCLUSIONS

1. *Planaria dorotocephala* of all ages used tolerate HCl up to about pH 4.9 and NaOH up to about pH 9.2 in the well water (pH = 7.5 to 7.6) in which they live, i.e., they tolerate a range of pH from 4.9± to about 9.2±.

2. Smaller, physiologically younger, individuals are on the average tolerant of a slightly wider range of hydrogen ion concentration (from pH = 4.7 to pH = 9.3) than are larger, physiologically older individuals, this difference of susceptibility being apparently somewhat greater on the acid than on the alkaline side of neutrality. The young possess a greater power of neutrality-regulation than do the old, explanatory suggestions for which are offered.

3. In concentrations of alkali which kill within a few hours susceptibility is reversed in relation to age, the young being very much more susceptible than the old. In similar concentrations of the acids young specimens are likewise on the average more susceptible, but only slightly more so. In other words, high concentrations of OH^- tend to increase and high concentrations of H^+ tend to diminish differences in direct susceptibility between young and old individuals. This suggests a possible increasing average acidity with senescence and decreasing metabolism.

4. Young planarians have about double the catalase content of old planarians per gram weight of tissue.

5. In acid solutions liberating CO_2, in which worms live for some time, they commonly assume, as in conditions of oxygen deficiency, a negative geotropism of an obviously adaptive nature as a normal means of escape from excess CO_2.

6. These facts indicate the necessity of taking into account the factors of age, size and metabolism in defining range of tolerance to agents and conditions.

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STUDIES ON THE ALKALINE RESERVE OF THE BLOOD OF THE INSANE

NOBUHARU SUITSU

From the Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania

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These studies were undertaken to ascertain whether or not the alkaline reserve of the blood of the insane shows any significant differences from that found in the blood of normal individuals, and whether or not the blood of the various types of insanity studied vary one from the other in their carbon dioxide combining capacity. During the course of the investigation certain other material was obtained which will be briefly discussed.

The subjects of these studies were patients at the Pennsylvania Hospital, Department for Mental and Nervous Diseases, Philadelphia.

Since Van Slyke, Stillman and Cullen (1) have shown that a slight rise in plasma carbon dioxide tension usually follows eating, the samples of blood to be analyzed were taken at the uniform hour of eleven o'clock in the morning, three and a half hours after breakfast, unless otherwise noted. The blood specimens were drawn from an arm vein with a Record syringe containing a small amount of potassium oxalate. Care was taken to avoid the sucking in of air. After filling the syringe the point of the needle was plunged under paraffin oil contained in a small test tube and 2 cc. of blood expressed and centrifuged. The plasma was then pipetted off and the carbon dioxide capacity determined as described by Van Slyke (2), and Van Slyke and Cullen (3). The determination was carried out two or three times on one and the same sample and the average adopted as the result.

A general comparison of the findings obtained from excited and depressed cases. It is well known that not only mechanical but also psychic activities have great influence upon respiratory and circulatory functions (1), and that the alveolar carbon dioxide tension under ideal normal conditions indicates the level of the blood bicarbonate, but since the alveolar carbon dioxide tension is altered by numerous factors, psychic, physiological and pathological (4), it is not a reliable measure of the blood bicarbonate except when it is certain that both the mechanical and nervous factors controlling respiration are normal.

With these facts in view an attempt was made to determine whether or not any relation existed between the plasma carbonate and conditions of excitement and depression. The figures in table 1 give the plasma CO_2 capacity in twelve cases classed as depressed and ten individuals exhibiting excitement.

It will be seen that in general terms all the values fall within normal limits and that no significant differences can be observed between the two groups. The mere fact that no differences occur in these two groups of patients permits the supposition that in long-continued excitations a compensatory reaction occurs sufficient to preserve the normal level of the alkaline reserve of the blood, which one would naturally suppose to be lowered by consequence of the increased activity.

TABLE 1

The alkaline reserve of the blood of the excited and depressed insane

Cubic centimeters of CO_2 reduced to 0°, 760 mm. bound as bicarbonate in 100 cc. plasma

EXCITED CASES	DEPRESSED CASES
64.42	72.08
64.42	69.14
63.95	66.36
63.57	66.36
63.48	66.36
62.08	63.54
60.20	59.74
54.38	59.68
51.24	57.39
45.04	55.94
	52.06
	51.18
Average........59.28	61.65

The alkaline reserve of the blood during individual changes in mental condition. A confirmation of the general findings that no evident differences are to be found in the alkaline reserve of the blood of excited or depressed insane patients is afforded by the figures given in table 2. These results represent the analyses of the bloods from single individuals at frequent intervals over periods of several weeks during which there occurred a marked change in mental condition with respect to excitement and relative depression. It is seen that in these cases at least there failed to occur any consistent change in the alkaline reserve of the blood accompanying the change from excitement to depression or vice versa. The four individuals studied were all diagnosed as dementia precox cases.

The variability of the alkaline reserve of the blood of the insane from week to week. During the course of these investigations opportunity was afforded to determine the degree to which the alkaline reserve of the blood varies in the individual from week to week in cases where the general trend of mental condition was uniform.

The results are given in table 3. The variability is given by the value for the average deviation calculated for each individual. An inspection of the table shows that although the absolute amounts all fall within normal limits and no significant differences occur, yet there are differences in the degree of variability to be observed in different individuals. These differences, however, cannot be evaluated since the data are insufficient.

Table 4 is a compilation of the analyses obtained from 112 bloods from 51 individuals arranged in the order of their descending value. The figures represent the cubic centimeters of CO_2 reduced to 0°, 760 mm. bound as bicarbonate by 100 cc. plasma. It is seen that the absolute amounts do not exceed the limits usually attributed to normal bloods save at the extremes. The main fact of interest lies in the relatively low variability of this blood factor which is comparable with that obtained for the average deviation of the blood creatinine nitrogen in similar patients (5).

In table 5 there have been arranged the values representing the average amounts, the average deviation, and the range of fluctuation of the alkaline reserve of the blood according to diagnosis. From the data it is evident that no significant differences obtain in the absolute amounts of the alkaline reserve of the blood in the different types here studied. It should be noted, however, that there is a tendency for the variability of this blood factor to be greater in the mentally disturbed than in normal individuals.

The alkaline reserve of the blood taken three and a half and fourteen hours after eating. Since it is often inconvenient to take samples of blood early in the morning and before breakfast a comparison was made of the alkaline reserve of the blood taken three and a half hours after breakfast, and fourteen hours after the night meal. The data are given in table 3.

TABLE 2

The alkaline reserve of the blood of four dementia precox patients during states of excitement and states of depression

NUMBER	CONDITION	CO$_2$ REDUCED TO 0° 760 MM. BOUND AS BICARBONATE IN 100 CC. OF PLASMA
		cc.
1	Quiet	50.24
	Excited	64.77
	Excited	63.30
	Quiet	59.68
	Depressed	60.56
	Excited	63.48
2	Excited	60.70
	Excited	63.43
	Depressed	68.51
	Excited	61.07
	Excited	74.80
3	Excited	63.30
	Quiet	60.19
	Quiet	57.78
4	Excited	56.71
	Quiet	54.22
	Quiet	52.49

TABLE 3

The individual variability of the alkaline reserve of the blood of the insane as observed from week to week

Cubic centimeters of CO$_2$ reduced to 0°, 760 mm. bound as bicarbonate in 100 cc. of plasma

	SET 1	SET 2	SET 3	SET 4	SET 5	SET 6	SET 7	SET 8	SET 9	SET 10	SET 11	SET 12
	58.46	55.34	53.21	70.07	64.30	65.47	62.60	64.12	74.90	59.80	69.20	72.96
	58.15	62.79	63.48	72.00	63.42	64.38	63.51	58.90	73.26	65.46	62.49	73.93
	66.73	62.40	62.56	75.38	65.30	57.74	65.50	59.80	70.10	62.58	66.79	64.38
	65.66	53.92	71.02	75.31	63.36	67.81	61.54	62.56	70.17	63.30	63.30	67.20
	63.42	66.31	63.66	74.85								
	63.44	64.34	63.42	73.57								
	69.14	70.41										
Average....	63.45	62.22	62.89	73.53	64.10	63.85	63.29	61.35	72.11	62.79	65.47	69.62
Variability.	4.0	7.0	5.3	2.3	1.2	4.8	1.9	2.7	2.8	2.3	4.4	5.5

TABLE 4

The carbon dioxide combining capacity of 112 bloods from 51 insane and normal individuals

ALKALINE RESERVE	DIAGNOSIS	ALKALINE RESERVE	DIAGNOSIS	ALKALINE RESERVE	DIAGNOSIS	ALKALINE RESERVE	DIAGNOSIS
75.38	M. D.	66.36	M. D.	63.42	M. D.	58.90	D. P.
75.31	M. D.	66.36	M. D.	63.42	N.	58.62	D. P.
74.90	D. P.	66.31	M. D.	63.42	I. M.	58.46	M. D.
74.85	M. D.	65.66	M. D.	63.36	N.	58.40	D. P.
74.80	M. D.	65.50	D. P.	63.30	D. P.	58.15	M. D.
73.57	M. D.	65.47	D. P.	63.30	D. P.	57.78	D. P.
73.26	D. P.	65.38	N.	63.17	D. P.	57.74	D. P.
72.08	M. D.	65.30	N.	63.09	D. P.	57.39	M. D.
72.00	M. D.	64.77	D. P.	62.79	M. D.	56.71	D. P.
71.04	N.	64.42	M. D.	62.60	D. P.	55.94	M. D.
71.02	I. M.	64.42	S. D.	62.56	D. P.	55.52	D. P.
70.41	M. D.	64.38	D. P.	62.56	I. M.	55.34	M. D.
70.17	D. P.	64.34	M. D.	62.44	M. D.	55.10	?
70.10	D. P.	64.30	N.	62.40	M. D.	54.38	M. D.
70.07	M. D.	64.12	D. P.	62.08	M. D.	54.22	D. P.
69.53	D. P.	63.95	M. D.	61.54	D. P.	53.92	M. D.
69.14	D. P.	53.66	I. M.	61.52	D. P.	53.21	I. M.
69.14	M. D.	63.61	N.	61.33	N.	52.97	D. P.
69.12	D. P.	63.57	N.	61.07	M. D.	52.75	M. D.
69.12	N.	63.57	M. D.	60.70	M. D.	52.65	?
68.60	D. P.	63.54	?	60.56	D. P.	52.49	D. P.
58.51	M. D.	63.54	D. P.	60.20	M. D.	52.06	D. P.
68.32	D. P.	63.54	N.	60.19	D. P.	51.24	D. P.
67.88	D. P.	63.51	D. P.	60.03	N.	51.18	?
67.81	D. P.	63.48	M. D.	59.80	D. P.	50.24	D. P.
67.22	N.	63.48	D. P.	59.74	I. M.	50.20	N.
66.73	M. D.	63.48	I. M.	59.68	D. P.	47.76	D. P.
66.36	M. D.	63.43	M. D.	59.68	D. P.	47.61	?

Average, 62.99; Variability, 7.35; Range, 73.38–47.61.

M. D., Manic depressive; D. P., Dementia precox; I. M., Involutional melancholia; S. I., Senile involution; N., Normal.

TABLE 5

The range of fluctuation, average amounts and average deviations of the alkaline reserve of the blood of the insane according to diagnosis

NUMBER OF CASES	DIAGNOSIS	RANGE	AVERAGE AMOUNT	AVERAGE DEVIATIONS
		cc.	*cc.*	*per cent*
15	Normal	47.61–71.04	62.60	6.6
39	Manic depressive	52.75–75.38	64.42	7.3
8	Involuntary melancholy	51.18–71.02	61.03	7.7
47	Dementia precox	47.76–74.90	61.84	8.2

Sets number 7–8–9 are the values obtained after the shorter fast, and sets 10–11–12 those found after the longer period of abstinence in the same individuals respectively. The investigation extended over eight weeks, the first four weeks of which being the period when the blood specimens were taken once a week after breakfast, and the second four weeks being the period when the samples were taken before breakfast.

It is evident that there are no valid or consistent differences in the bloods taken at these times.

SUMMARY

The results of the studies here reported indicate that:

1. The alkaline reserve of the blood of the insane appears to fall within the limits considered normal for healthy persons.
2. There are no demonstrable differences in the absolute amounts of the alkaline reserve of the bloods from excited or depressed patients here studied.
3. The variability of the plasma carbon dioxide combining capacity seems to be higher in the insane than in the small group of normals here studied.
4. No noteworthy differences obtain in the alkaline reserve of the blood taken three and a half and fourteen hours after eating.

I take this occasion to express my appreciation of the courtesy of Dr. Owen Copp in affording me the facilities of the Pennsylvania Hospital, Department of Nervous and Mental Diseases. The work was carried on under the direction of Dr. Frederick S. Hammett, for whose help and advice I am deeply grateful.

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GASTRIC TONUS OF THE EMPTY STOMACH OF THE FROG

COMPARATIVE STUDIES IV[1]

T. L. PATTERSON

From the Hull Physiological Laboratory, The University of Chicago and the Physiological Laboratory, Queen's University

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Sherrington (1) in 1915 called our attention to the reflex postural activity of muscle and nerve as being the main outcome of the functioning of the proprioceptive part of the nervous system for at least the skeletal muscle. He pointed out that the muscle fiber possessed the property of exhibiting different lengths while exhibiting one and the same degree of tension, and that it was not to be regarded as an elastic band. Furthermore, he believes that unstriated muscle, like skeletal muscle, possesses the same properties as is shown by the ease with which the hollow visceral organs, like the bladder and stomach, adapt their size to the volume of their contents and with very little alteration in their intravesical pressure. Under these conditions, visceral tonus is therefore postural configuration. In confirmation of this Hurst (2) found that the relaxation of the rectum was analogous to what Sherrington described as the "lengthening reaction" of the "postural tone" in the skeletal muscles and in the bladder, and which he at an earlier date had described in connection with the stomach and intestine, although he had not actually used the expression "visceral tone." In case of the skeletal muscle the reflex postural action depends normally upon the afferent nerve of the posturing muscle itself, while in the unstriated muscle it is far less dependent on the central nervous system for its adjustment and maintenance.

More recently Grey (3) has shown by slowly filling the empty viscus with warm physiological saline solution and recording the fluctuations in the intragastric pressure that the normal stomach in rabbits and cats is capable of adapting its size to the volume of its contents with very small changes in the intragastric pressure. According to this investigator, the mechanism involved in the postural configuration of the stomach is situated in the wall of the viscus itself and concerns solely its musculature together with its intrinsic nervous mechanism, while the extrinsic nerves exhibit no direct influence, but serve rather to regulate the tension of the stomach wall.

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[1] A preliminary report of this work was made before the 1919 meeting of the American Physiological Society at Baltimore, a brief abstract of which was published in the Proceedings of that society.

The experiments summarized in this report were undertaken with the view of securing further data on the gastric tonus (postural activity —Sherrington, Hurst, Grey) of the neuro-muscular apparatus as applied to the empty stomach. While the term "postural activity" is very applicable to the skeletal musculature it appears to me that it is not well suited for the unstriated musculature which makes up the larger portion of the walls of the hollow visceral organs, therefore the older and simpler terminology of gastric tonus will be used throughout this paper. The results tend to show that the extrinsic nerves exert a partial influence on the tonal activity of the stomach viscus, as well as serving to modify and regulate the gastric activity at least in the frog. This animal is particularly adapted for such a study for it has been shown in a previous paper (4) of this series that the gastric hunger contractions show no periodicity and no appreciable change in gastric tonus, both features of which are present in the higher animals. In contradistinction to the higher animals, the contractions are practically continuous with scarcely any distinction between the digestive peristalsis and the hunger movements.

Among the first to make observations upon the internal pressure of the hollow visceral organs were Mosso and Pellacani (5) who investigated the bladder in man and in the dog. These authors found that the bladder is capable of adjusting its cavity-volume to different quantities of content, which it enfolds with about the same light tension of grasp whether the viscus is nearly empty or well filled. Somewhat similar observations have been made upon the fundic portion of the stomach. Kelling (6) found that within certain limits the intragastric pressure remained unaffected by the quantity of fluid within the viscus and that the intra-abdominal pressure altered very little in the dog before and after the taking of a copious meal, although the intake of the volume of food might amount to 50 per cent of the total contents of the abdomen in the fasting condition. He infers from these latter observations that the additional volume of contents must be accommodated for by a reflex adjustment of the postural contraction of the abdominal muscles. Pike and Coombs (7) in confirmation of the above have reported that the introduction of fluid into the stomach or into the peritoneal cavity of cats causes lengthening of the rectus abdominis muscle while the flow of fluid out of the stomach causes a shortening of the same muscle. These changes in the length of the muscle are small and do not occur if the posterior roots of the spinal nerves supplying the muscle have been cut, or if the spinal cord has been transected at the level of the lower cervical roots. The section of both vagi has no marked effect on the response of the muscle. The authors regard the change in the length of the muscle corresponding to the increase or decrease in volume of the contents of the abdominal cavity as a reflex process dependent upon afferent impulses which falls into line with other known instances of postural activity of muscle and nerve. The observations of Sick and Tedesko (8) and others have shown that the gradual filling of a cat's stomach is not accompanied by a rise in intragastric pressure and that the excised stomach, kept alive in a bath of warm oxygenated Ringer's solution also exhibits the same phenomenon to an unmistakable extent.

Cannon and Lieb (9) have also brought forth evidence that each passing of the cardia by swallowed food is accompanied by a rapid small dilatation of the fundus, and that this dilatation is a reflex operated through the vagus. Rogers (10) has reported that central stimulation of one vagus nerve with the opposite nerve intact in the decerebrate dog and after complete splanchnic section leads to reflex spasmodic contractions of the entire stomach and increased gastric tone. Therefore it would appear that the adaptability of the normal stomach at all times is a form of receptive expression brought about by changes in the intragastric pressure as the volume of its contents slowly increases or decreases.

Experimental procedure. The same general method of experimentation was used in the following experiments on the bullfrog (Rana catesbiana) as that described in the preceding paper (11) of this series, with the exception that the gastric balloon was inflated with a known quantity of air by means of a graduated glass syringe sufficient to maintain a constant pressure of 2 cm. in the water manometer and the number of cubic centimeters of air necessary in the different experiments to produce this constant pressure was recorded. Normal contractions of the empty stomach were obtained from each animal, extending over a period of several days, and then these animals were either vagotomized, splanchnetomized or vago-splanchnetomized (section of both sets of nerves) and the respective observations repeated over a period of from two to three weeks and compared with the normal. The recorded tracings were taken on a slowly moving drum making a revolution in fifty to sixty minutes.

The changes in volume capacity of the stomach as influenced by partial and complete isolation from the central nervous system. The influence of the vagi and splanchnic nerves on the activity of the empty stomach of the frog has been reported in a previous paper (11). According to these results, double vagotomy leads to a sympathicotonic condition of the stomach followed with nearly the normal type of hunger contractions with the exception that they appear to be of a somewhat slower rate and slightly weaker. On the other hand, section of the splanchnic nerves leads to a hypertonic stomach with shallow contractions, showing an increased rate and tending to run into incomplete tetanus, while complete isolation of the stomach from the central nervous system leads to a hypotonic stomach with about the normal type of gastric hunger contractions. Somewhat similar changes have been described by Cannon (12) on cats for the digestive movements and by Carlson (13) on dogs for the movements of the empty stomach.

Although the sectioning of these nerves in various animals has led to certain changes in gastric tonus, as arising from the influence exerted through the extrinsic nerves supplying the stomach, no attempt has been made to analyze the question quantitatively. In order to study the changes in volume capacity of the empty stomach as influenced by partial and complete isolation from the central nervous system, twenty-one animals were used for the various observations recorded herein, as follows,—seven were vagotomized, seven splanchnetomized and seven vago-splanchnetomized. In addition, fifteen other animals were used but as the length of the duration of these experiments was more or less brief due to parasitization or other causes leading to an early death, the data from these were excluded. However, in none of these experiments in which results were obtained were they contradictory to the typical results as tabulated. There were also a few animals of this number excluded because of incomplete nerve section. The following tables have been prepared as showing typical results of the experiments.

The animals used for the observations in the preceding experiments were twelve to thirteen inches in length, extended, and it was found, without exception in this size of animal, that 10 cc. of air introduced by a syringe into the gastric balloon was sufficient to maintain a constant manometric pressure of 2 cm. in the stomach of the normal animal. Larger animals in proportion to size require greater quantities of air to obtain this constant manometric pressure, and vice versa. In one case, a very large frog measuring sixteen inches, the only one used in the series of experiments, 15 cc. of air were necessary to produce the constant pressure of 2 cm. in the water manometer. The question of the elasticity of the rubber balloon may arise here for, as Osborne (14) pointed out, in thin-walled rubber bags the extensibility of the elastic material is great and its dimensions, including its thickness, alter much under the stretch imposed. Furthermore, a subspherical

TABLE 1

*Effect of section of the vagus nerves on volume capacity of stomach and contractions**

DATE 1918	CONDITIONS	AIR IN BALLOON	STRENGTH OF CONTRACTIONS	REMARKS
		cc.	*cm.*	
August 7	Stomostomized			
August 10	Normal	10	6.5	
August 11	Normal	10	6.0	
August 12	Normal	10	6.5	
August 13	Vagotomized			Operation O.K.
August 16	Vagotomized	15	6.5	
August 17	Vagotomized	15	8.0	
August 18	Vagotomized	15	6.8	
August 19	Vagotomized	15	7.0	
August 20	Vagotomized	15	8.0	
August 21	Vagotomized	15	6.5	
August 22	Vagotomized	13	6.5	
August 23	Vagotomized	10	6.0	
August 24	Vagotomized	10	4.5	
August 25	Vagotomized	10	5.5	
August 26	Vagotomized	10	5.8	
August 27	Vagotomized	10	6.2	
August 28	Vagotomized	10	5.0	
August 29	Vagotomized	10	4.0	
August 30	Vagotomized	10	3.5	
August 31	Vagotomized	10	Very weak	
September 1	Vagotomized	10	Very weak	
September 2	Vagotomized			Animal died. Autopsy showed both vagi cut

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* Work now in progress on lung tonus of the frog shows that double vagotomy leads to practically the same effects as extirpation of the lungs, and this may shorten the life of the animals.

bag may change in general figure as its size is altered, or changes in the physical consistence of the rubber membrane may occur as inflation and deflation proceeds, all of which would lead to serious complication for the analysis of results. However, in the case of the gastric balloon used 10 or even 15 cc. of air do not fill the rubber balloon, so that the tension of the bag's elasticity complicates the stomach tonus.

TABLE 2

Effect of section of the splanchnic nerves on volume capacity of stomach and contractions

DATE 1918	CONDITIONS	AIR IN BALLOON	STRENGTH OF CONTRACTIONS	REMARKS
		cc.	cm.	
August 7	Stomostomized			
August 10	Normal	10	6.0	
August 11	Normal	10	6.7	
August 12	Normal	10	6.5	
August 13	Splanchnetomized			Operation O.K.
August 16	Splanchnetomized	4	0.5	
August 17	Splanchnetomized	4	1.0	
August 18	Splanchnetomized	4	0.8	
August 19	Splanchnetomized	4	0.6	
August 20	Splanchnetomized	4	0.5	
August 21	Splanchnetomized	4	1.4	
August 22	Splanchnetomized	6	0.5	
August 23	Splanchnetomized	10	0.3	
August 24	Splanchnetomized	10	0.4	
August 25	Splanchnetomized	10	0.3	
August 26	Splanchnetomized	10	0.3	
August 27	Splanchnetomized	10	0.2	
August 28	Splanchnetomized	10	Very weak	
August 29	Splanchnetomized	10	Very weak	
August 30	Splanchnetomized	10	Very weak	
August 31	Splanchnetomized			Animal died. Autopsy showed splanchnics cut

In testing out the amount of air necessary to produce the constant manometric pressure it was found without exception in all the animals that a much smaller amount than 10 cc. of air would produce changes in the manometer amounting to 2 or more centimeters but the length of its duration was very short and the pressure soon fell to the zero level or closely approximated it depending on the quantity of air introduced. This is indicative of the ease with which the stomach adapts its size to the volume of its contents.

In female animals filled with large egg masses the number of cubic centimeters of air necessary to produce the constant pressure showed no variations from that of the non-egg-carrying female and the male, although the abdomen was much enlarged. This condition in the egg-carrying female is doubtless accounted for, at least in part, by a reflex mechanism leading to a relaxation of the abdominal muscles, an adaptation similar to the reflex relaxation of the rectus abdominis muscle in increased volume contents of the stomach as has been described by

TABLE 3

Effect of section of the vagi and splanchnic nerves on volume capacity of stomach and contractions

DATE 1918	CONDITIONS	AIR IN BALLOON	STRENGTH OF CONTRACTIONS	REMARKS
		cc.	*cm.*	
October 22	Stomostomized			
October 25	Normal	10	6.5	
October 26	Normal	10	6.5	
October 27	Normal	10	6.9	
October 28	Normal	10	6.7	
October 28	Vagi and splanchnics cut			Operation O.K.
November 2	Vagi and splanchnics cut	15	6.7	
November 3	Vagi and splanchnics cut	15	7.0	
November 4	Vagi and splanchnics cut	15	6.9	
November 5	Vagi and splanchnics cut	15	7.2	
November 6	Vagi and splanchnics cut	15	6.6	
November 7	Vagi and splanchnics cut	15	8.0	
November 8	Vagi and splanchnics cut	15	6.5	
November 9	Vagi and splanchnics cut	15	5.8	Morning
November 9	Vagi and splanchnics cut	13	6.0	Night
November 10	Vagi and splanchnics cut	13	5.0	
November 11	Vagi and splanchnics cut	13	4.4	
November 12	Vagi and splanchnics cut	13	3.5	
November 13	Vagi and splanchnics cut	13	3.4	
November 14	Vagi and splanchnics cut	13	3.5	
November 15	Vagi and splanchnics cut	13	Very weak	
November 16	Vagi and splanchnics cut	13	Very weak	
November 17	Vagi and splanchnics cut	13	Very weak	
November 18	Vagi and splanchnics cut			Animal died. Autopsy showed both vagi and splanchnics cut

Pike and Coombs (7). The animals with very few exceptions were run continuously as soon as recovery was complete after the operation and the fast commenced immediately.

Section of both vagi or the vago-sympathetic nerves (11) in the neck of the frog increases the volume capacity of the stomach temporarily, as shown in table 1, from the normal of 10 cc. to 15 cc. of air. This condition invariably lasts from eight to nine days. Usually on the ninth day following the cutting of the nerves there is a decrease in the

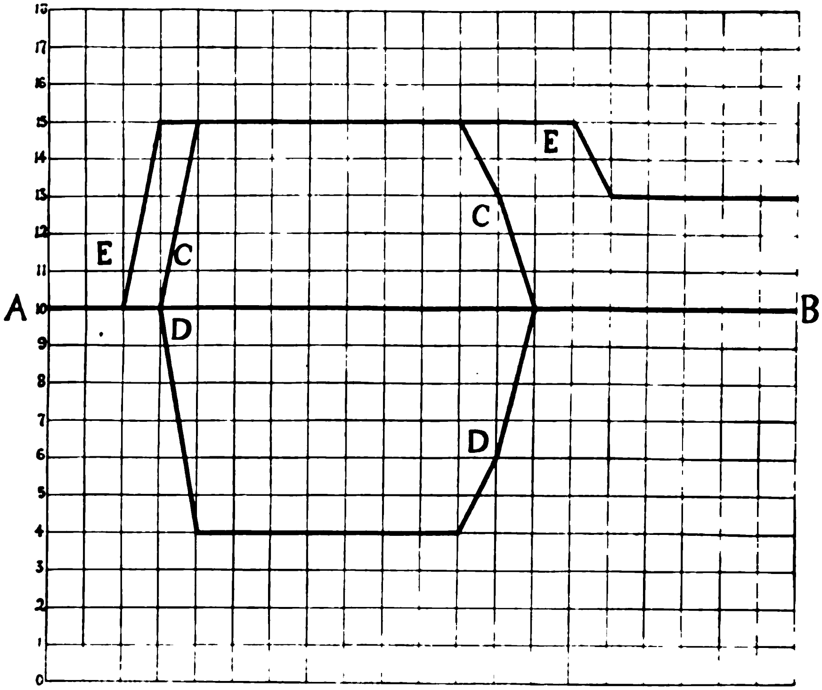

Fig. 1. The spaces left to right indicate the number of days experiment ran. Vertical spaces above and below the heavy line *A B*, representing the normal pressure of 10 cc. of air necessary to maintain a constant pressure of 2 cm. in the water manometer, indicates the positive or negative changes from the constant in the volume capacity of the stomach as influenced by the extrinsic nerves. Curve *C C*, shows effect of sectioning both vagi on stomach. Curve *D D*, effect of splanchnic section. Curve *E E*, combined effect of section of both vagi and splanchnic nerves. Note complete recovery of gastric tonus in first two cases, while in the latter there is only a partial recovery. Heavy line *A B* also indicates negative effect of decerebration on stomach. Figures at left indicate number of cubic centimeters of air in balloon.

intragastric pressure to about 13 cc. of air and on the next day it drops again to the normal or 10 cc. level, and remains there. In other words, the normal tone of the stomach has been reëstablished (fig. 1), and this condition as it exists in the frog may be comparable to the temporary loss of tonus as described by Cannon (12) in cats. The contractions of the empty stomach tend to approach the normal, but on the whole they are of a slightly slower rate and more irregular. The amplitude of the individual contractions may even appear greater than normal and this may be because the contractions start rather suddenly and without any marked preliminary increase in tonus in the fundic end of the stomach.

Section of the splanchnic nerves in the frog markedly decreases the volume capacity of the stomach temporarily, as shown in table 2, from the normal of 10 cc. to 4 cc. of air. This marked diminution in size like the increase after double vagotomy invariably lasts from eight to nine days. Usually on the ninth day following the cutting of the nerves there is an increase in the intragastric pressure to about 6 cc. of air, while on the next day it reaches again the normal or 10 cc. level and remains there. Here again the stomach has reëstablished its gastric tonus (fig. 1). This condition in the frog is much more marked than Carlson (13) found it to be in dogs for just as the number of cardioinhibitory fibers vary in the vagus of the cat and the dog, so also may not the number of motor fibers in the vagi destined for the stomach vary in different animals? The contractions of the empty stomach are small, showing an increased rate and a tendency to approach incomplete tetanus. This is especially true during the temporary period of high tonal activity when only 4 cc. of air are required to maintain the constant manometric pressure and in one animal 3 cc. of air were found to be sufficient. In a few such animals I have found in the morning following the removal of the balloon the night before such strong gastric and esophageal contraction that it was impossible to introduce the balloon through the short esophagus into the stomach without first introducing a small glass seeker and stretching it. I have even had difficulty in introducing the seeker the first time on one or two occasions because of such marked contraction. This would seem to uphold the views of Cannon (12) and Kelling (6) that the gastric fibers of the vagi function to make the gastric muscles exert a tension.

Section of the vagi and splanchnic nerves in the frog increases the volume capacity of the stomach permanently, as shown in table 3, from the normal of 10 cc. to 15 cc. of air, but in this case there is not a complete recovery. After this complete isolation of the frog's stomach from the central nervous system, the 15 cc. stomach invariably lasts from twelve to thirteen days, which is a longer period than in either the vagotomized or splanchnetomized stomach. Usually on the thirteenth day following the sectioning of these nerves which is accomplished at one operation there is a fall in the intragastric pressure to a 13 cc. level, where there are no further changes (fig. 1). This new and partial readjustment of the hypotonic stomach is evidently determined by the intrinsic local gastric motor mechanism of the stomach wall for the gastric hunger contractions persist after its isolation from the central nervous system. The appearance of the individual contractions is much the same as when the vagi alone are cut. These contractions may exhibit a greater or lesser amplitude and show a tendency toward irregularity. All the animals in the different groups were autopsied to verify more especially the sectioning of the respective nerves. In a few of these animals in which the heart was still beating regularly the effect of vagal stimulation on the stomach was determined. This resulted usually in a phase of inhibition followed by a stronger phase of excitation immediately upon the removal of the stimulus and is in confirmation with the findings of Hopf (15) on frogs. Stimulation of the sectioned splanchnic usually resulted in a relaxation of the body of the stomach, if any change at all occurred, and if the stimulation was repeated several times in succession it seemed to bring about a constriction of the pyloric sphincter and perhaps also that of the cardiac sphincter, a condition which would seem to indicate that the splanchnics might possess a few fibers of the motor type. The stimulation of these two nerves does show, however, that the fibers of neither have degenerated, and since the rate of nerve degeneration differs in different animals and in frogs requires from thirty to one hundred and forty days, depending upon the season of the year (16), there is no possibility of the regeneration of these nerves. The normal functioning of the two sets of nerves to the stomach is indicated by the results of sectioning, as well as by the results of stimulation. In the case of the isolation of the stomach from the influence of the vagi with the splanchnics intact, or vice versa, there is a perfect physiological readjustment of the normal tonus of the gastric musculature. On the other hand, after complete isolation of the stomach (vagi and splanchnics severed) from the central nervous system there is only a partial physiological readjustment of the gastric musculature. This indicates that the extrinsic nerves play a prominent part in the maintenance of gastric tonus, at least in the frog. When the splanchnics are sectioned it must be the motor fibers of the vagi that produce the high and temporary gastric hypertonicity, i.e., hypertonic stomach. When the vagi are cut and the splanchnics permitted to exert their full influence on the gastric musculature it is reasonable to believe that these nerves must possess motor fibers probably to the sphincter muscles and that these areas then act as tonic rings which, in connection with the intrinsic or local reflex mechanism of the gastric wall, are capable of producing a perfect physiological readjustment. Whereas, in the case of the stomach completely isolated from the central nervous system, this intrinsic or local gastric mechanism is incapable of bringing about a complete readjustment and in consequence of this it creates a new level of gastric tonus. Thus, every reflex is in its own measure an integral reaction, and is purposive in that it bears some biological purport for its organism. This physiological readjustment occurred regularly in all the animals, that is, it could be looked for after a lapse of a certain number of days following the sectioning of the nerves. For example, in the case of the vagotomized stomach when this readjustment started I have seen in a few instances the pressure in the manometer increase from the constant level of 2 cm. to 5 or 6 cm., but I have never observed it in the stomach of the normal animal. In the splanchnetomized stomach of the course the first readjustment stage is marked by a fall in the manometric pressure to zero.

The changes in gastric tonus observed throughout this series of experiments are so slight in the normal frog that they are practically unmeasurable. However, tonus is the prime condition for that tension which must be developed before contraction can result and if the tension persists the contraction recurs (17). Furthermore, the importance of the tonic state in the normal functioning stomach is reinforced by the fact that when all the extrinsic nerves are cut the stomach develops in time within itself a tonic state, while the adaptability of the abdominal cavity to the volume of its contents is left to the postural reflex.

The effect of decerebration on the tonus of the stomach. It has been shown by King and Connet (18) that the rate of the gastric contractions is increased in decerebrate guinea pigs and that the stomach becomes hypertonic. According to Rogers (19) the hyperactivity of the crop of the decerebrate pigeon is inhibited by food and water as in the normal bird, while the writer (4) has reported no change in the type of the contractions from the empty stomach of the normal and the decerebrate frog. In order to study the effect of decerebration on the volume capacity of the stomach, observations were made on six decerebrate frogs. The following table has been prepared from a typical experiment.

Decerebration in the frog has no effect on either the volume capacity of the stomach or the amplitude of the individual contractions, as is shown in table 4. There is also no change in the type of contractions, which confirms the work of the previous paper (4). The negative findings in these experiments show that the higher cerebral centers in the frog play no appreciable part in either the maintenance of gastric activity or the tonic state. Since section of the vagi leaves the stomach in a temporary hypotonic condition (15 cc. stomach) while the decerebration effects are negative we may infer that impulses from centers in the mid-brain and medulla exercise the controlling influence and produce after section of the splanchnics the temporary hypertonic stomach, i.e., high gastric tonus. It may be further implied that there is a dynamic readjustment in the central nervous system which leads to an actual diminution in the inhibitory impulses through the splanchnics after vagal section or an inverse motor condition existing through the vagi after splanchnic section, or else the stomach may bring about its physiological readjustment by an increased resistance or tolerance of the splanchnic or motor impulses over the respective nerves to the gastric mechanism.

TABLE 4

Effect of decerebration on volume capacity of stomach and contractions

DATE 1918	CONDITIONS	AIR IN BALLOON	STRENGTH OF CONTRACTIONS	REMARKS
		cc.	*cm.*	
July 26	Stomostomized			
July 31	Normal	10	6.0	
August 1	Normal	10	6.5	
August 2	Normal	10	6.0	
August 2	Decerebrated 4:15 p.m.			Operation O.K.
August 2	Decerebrated	10	6.0	Contractions started again at 5:35 p.m.
August 3	Decerebrated	10	6.3	
August 4	Decerebrated	10	6.0	
August 5	Decerebrated	10	5.2	
August 6	Decerebrated			Animal died. Autopsy showed complete removal of cerebral hemispheres

CONCLUSIONS

1. The normal stomach of the frog possesses a marked capacity for adapting itself to the volume of its contents with only minimal changes in the intragastric pressure. This is in confirmation with the work of Grey and others.
2. Both the intrinsic and extrinsic nerves take part in the maintenance of gastric tonus as is shown by partial and complete isolation of the stomach from the central nervous system.
3. Section of the vago-sympathetic nerves (double vagotomy) with the splanchnics intact increases the volume capacity of the stomach temporarily, but there is later a complete readjustment.
4. Section of the splanchnic nerves with the vagi intact decreases the volume capacity of the stomach temporarily, but there is again a complete readjustment as above.
5. Section of both the vagi and splanchnic nerves (complete isolation from the central nervous system) increases the volume capacity of the stomach permanently, and in this case there is only a partial readjustment, at least for a period extending over three weeks, and the tonus of the stomach is established upon a new level from that of the normal.
6. Decerebration affects neither the volume capacity of the stomach nor the type of the contractions.

The writer desires to acknowledge his indebtedness to Doctor Carlson for his kindly and valuable criticism.

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STUDIES ON THE SUBMAXILLARY GLAND

VI. On the Dependence of Tissue Activity upon Volume-flow of Blood and on the Mechanism Controlling this Volume-flow of Blood

ROBERT GESELL

From the Departments of Physiology of Washington University Medical School and of the University of California

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INTRODUCTION

This paper has to do with two problems: one the dependence of tissue activity upon volume-flow of blood, and the other the mechanism by which the volume-flow of blood is controlled. While these problems may be considered as distinct from each other, yet they have a certain interdependence which may warrant their discussion in common.

I have previously reported results bearing upon both problems (1), (2). Although no definite conclusions were reached concerning the mechanism of volume-flow control, it was shown that in hemorrhage the organism as a whole suffers from a reduced flow of blood as is indicated by the reduced alkaline reserve of the plasma of the blood. But despite the fact that the organism suffers from a reduced flow of blood we know from the work of others (3), (4) that a tissue may be stimulated to great activity even though the flow of blood may be very low or even absent. This apparent independence of volume-flow and tissue activity is shown in figures 1 and 3, where secretion of saliva is used as the index of tissue activity.

DEPENDENCE OF SECRETION UPON VOLUME-FLOW OF BLOOD

To show the dependence of tissue activity upon volume-flow of blood, using secretion as the index to activity, special methods must be employed. The greatest care must be taken that change in volume-flow of blood be the only variable. When the gland is activated by stimulation of the chorda tympani the periods and strength of stimulation must be equal and periods of rest must be chosen which will avoid the augmenting effect of previous stimulation. Results obtained under such conditions can be compared with results obtained when the blood supply is modified. Such results are shown in figures 1, 2 and 3, in which blood pressure, volume-flow of blood, secretion, electrical deflections, time in seconds and moment of stimulation of the chorda tympani are recorded. The volume-flow of blood was measured with the bloodless method previously described.

Figure 1, *A* and *B*, shows the effects of occluding the carotid artery during a short period of stimulation lasting 14 seconds. In the first record, the artery was unoccluded and stimulation of the chorda tympani produced the usual rapid acceleration of blood flow. In the second, where the artery was occluded, the flow of blood during the period of stimulation was slow but the amount of saliva secreted was not diminished. The results might be taken to indicate that even large fluctuations in volume-flow of blood need not affect the metabolic processes of the gland, were it not for the change in the contour of the electrical deflections which suggests that the glandular processes were modified. On the other hand, the absence of an after-flow of blood following de-occlusion indicates that the gland was not overtaxed by the temporary reduction in the blood-flow.

Figure 1, *C* and *D*, shows the effect of occlusion of the artery during greater activation. The secretion of saliva during occlusion was not reduced, yet the electrical deflection was modified again as in 1, *B*. The difference between the results shown in 1, *A* and *B*, and in 1, *C* and *D*, is that de-occlusion in the latter observations was followed by a markedly accelerated after-flow of blood indicative of an overstrain of the tissue resulting from activation without sufficient blood supply.

Figure 2, *A*, *B* and *C*, shows the effect of more prolonged occlusion of the artery lasting through a period of stimulation of 30 seconds. Even this longer period of occlusion has little effect upon the amount of saliva obtained—16.2 drops during the period of occlusion as compared with 18.2 and 19.2 drops during the preceding and subsequent periods of free flow of blood. In figure 3, *A*, *B*, *C* and *D*, where the periods of stimulation and occlusion were still longer, the reduced flow of blood again had relatively little effect. In fact (*C*) during occlusion 18.4 drops of saliva were secreted compared with 19.0, 19.6 and 20.3 in the preceding and subsequent periods.

Upon the whole, the results indicate that relatively short periods of occlusion of the carotid artery affect little the amount of saliva elicited by stimulation of the chorda tympani. This absence of marked effects may be apparent only and may be due to the fact that the gland at the moment of stimulation has recovered from previous activation and readily liberates its stored material and energy regardless of the momentary decrease in flow of blood during the period of activation. If so, it would be better to study the dependence of tissue metabolism

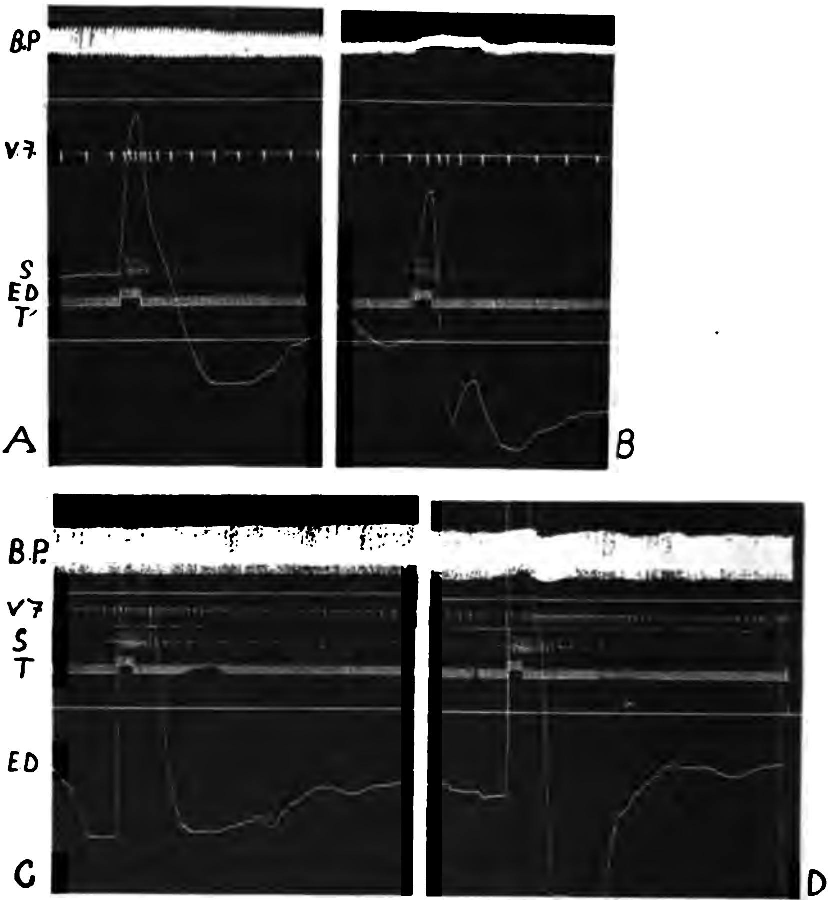

Fig. 1. Effect of occlusion of the carotid artery on the response of the submaxillary gland to stimulation of the chorda tympani. 1*A*, normal; 1*B*, artery occluded; 1*C*, normal; 1*D*, artery occluded; *B.P.*, blood pressure; *V.F.*, volume-flow of blood; *S.*, salivary secretion; *E.*, electrical deflection; *T.*, time in seconds and moment of stimulation of the chorda tympani.

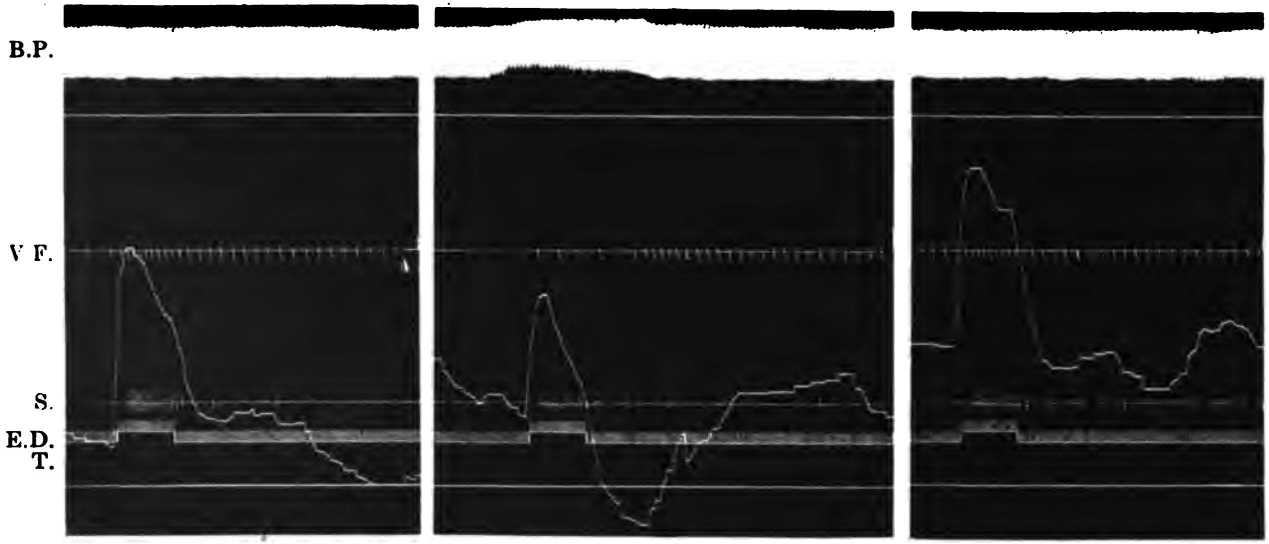

Fig. 2. Effect of occlusion of the carotid artery on the response of the submaxillary gland to stimulation of the chorda tympani. *2A*, normal, 18.2 drops of saliva secreted; *2B*, artery occluded, 16.2 drops of saliva secreted; *2C*, normal, 19.2 drops of saliva secreted.

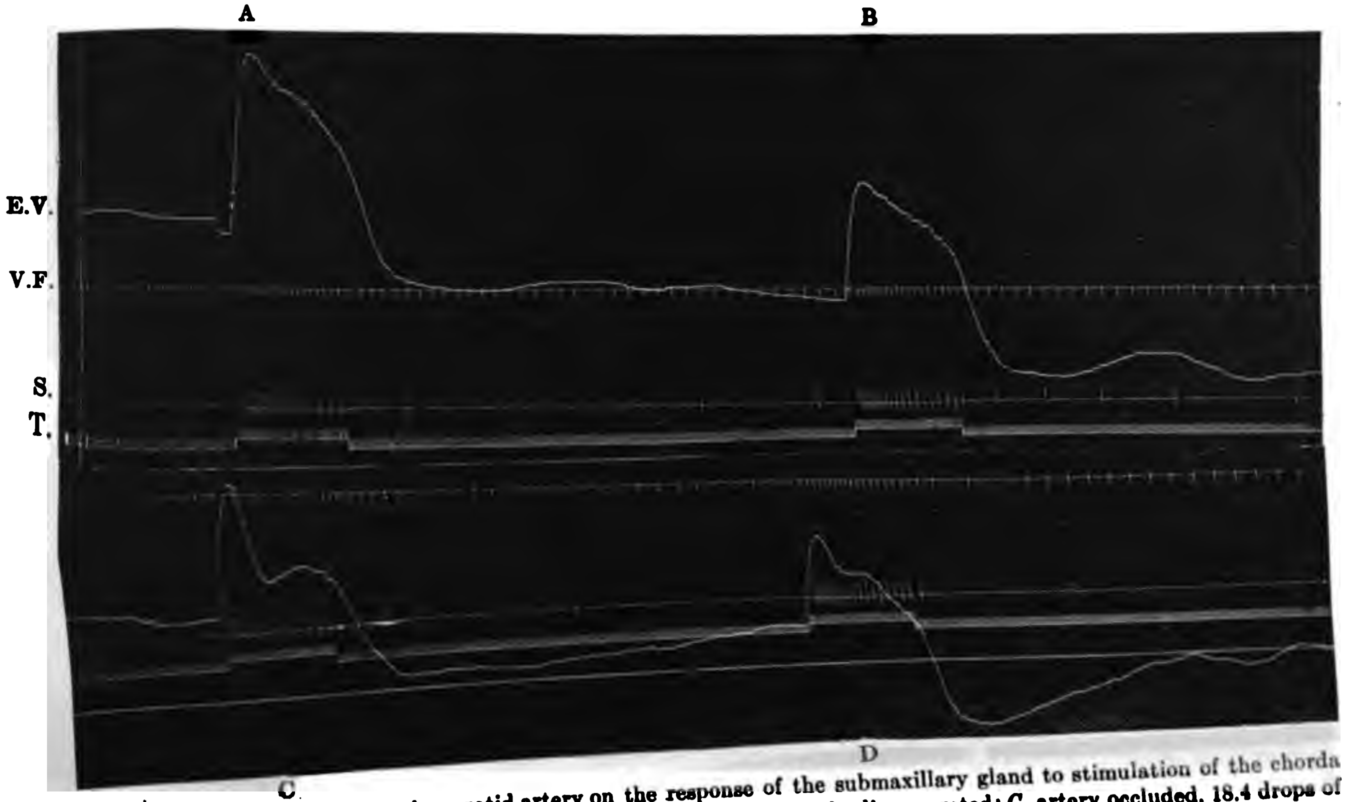

Fig. 3. Effect of occlusion of the carotid artery on the response of the submaxillary gland to stimulation of the chorda tympani. *A*, *B* and *D*, normal records with 19.0, 19.6 and 20.3 drops of saliva secreted; *C*, artery occluded, 18.4 drops of saliva secreted.

upon tissue which has not fully recovered from previous activation; for this purpose the gland might be activated over a longer period of time, and the blood supply modified during this period of activation, that is while activity and recuperation are going on hand in hand.

A series of such experiments is shown in figure 4, *A*, *B*, *C*, *D* and *E*. In figure 4, *A*, *B* and *C* the gland was activated by the injection of pilocarpin and the flow of blood was restricted in three ways: in *A* by obstruction of the carotid artery; in *B* by injection of adrenin; and in *C* by stimulation of the vago-sympathetic. In every instance the slowing of the flow of blood affected the response of the gland to the stimulation of pilocarpin. The objection might be raised that we not only interfered with the blood supply but also with the supply of pilocarpin which stimulates the gland. This objection can not hold in the experiment represented in figure 4, *D* and *C*, where the gland was activated by stimulating the chorda tympani. In 4, *D*, the flow of blood was decreased during stimulation by occluding the artery. The volume-flow of blood was not recorded but the moments of occlusion and de-occlusion are evident in the blood pressure tracing. It will be noted that the effect of occlusion became progressively greater as occlusion continued, suggesting that stored up saliva may be easily liberated, whereas the storage and liberation of new saliva required a greater flow of blood. The slowing of the secretion is undoubtedly due to the slowing of the blood stream and not to fatigue of the gland from prolonged stimulation, as is indicated by the subsequent acceleration of secretion upon de-occlusion of the artery. In this record secretion continued for some time after cessation of stimulation. Occlusion of the artery during this after-secretion again retarded secretion. The effects of injection of adrenin during prolonged stimulation of the chorda tympani were quite as striking—see figure 4, *E*.

The gland from which figure 5 was obtained was extremely sensitive to changes in volume-flow of blood. The period of stimulation of the chorda tympani lasted from *A* to *B*. The close parallelism between secretion and volume-flow of blood is very evident. Short irregular fluctuations which were not due to occlusion of the artery also are to be seen.

Figure 6 is taken from the same experiment as figure 5. It shows again the effect of volume-flow of blood upon the threshold of stimulation for secretion. Stimulation of the chorda tympani elicited an increased volume-flow of blood and though no visible secretion occurred it excited the gland, as is evidenced by the electrical deflection. That

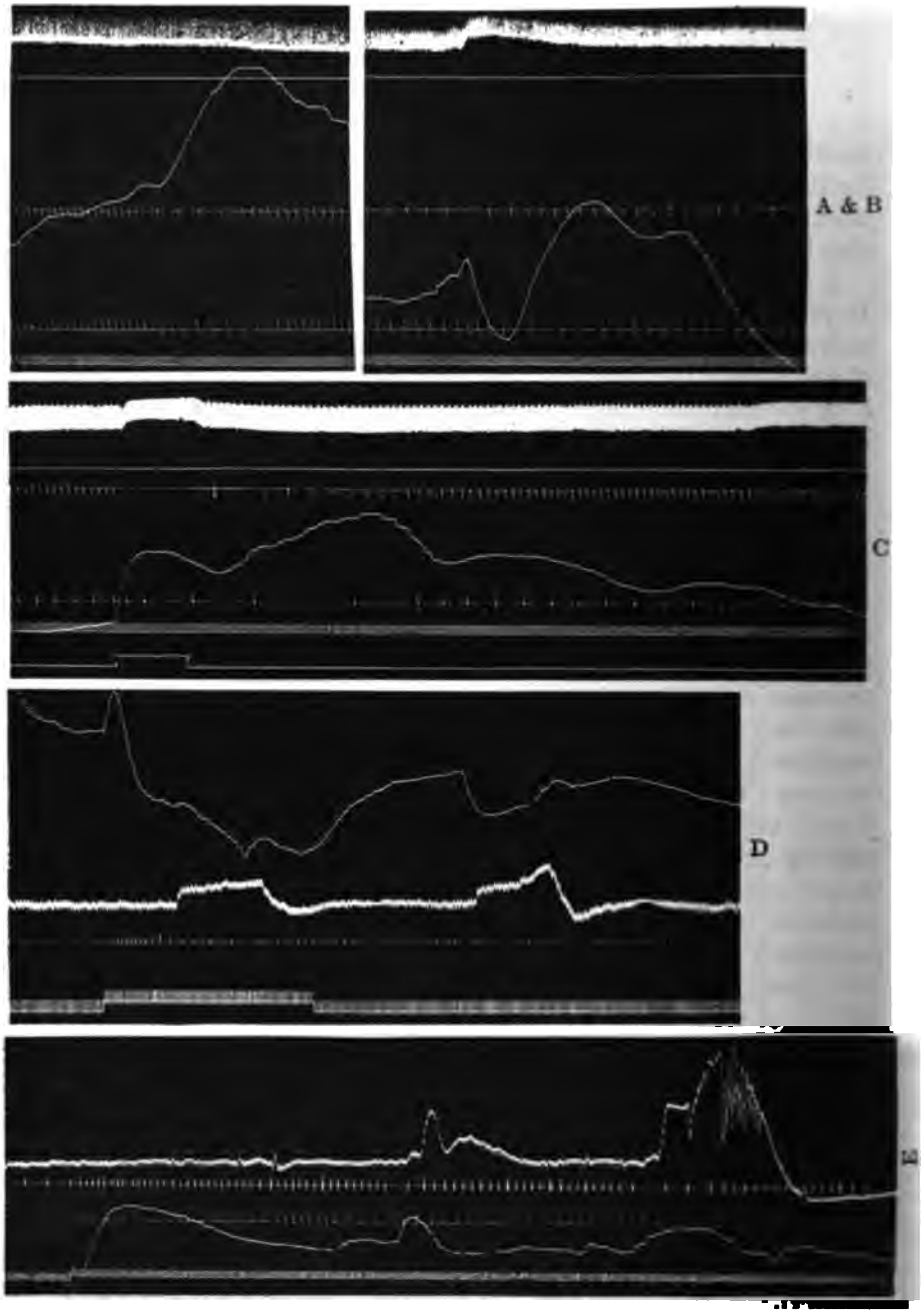

Fig. 4. Effect of interfering with the blood supply to the submaxillary gland during prolonged activity. In *A*, *B* and *C*, the gland was activated by the injection of pilocarpin and the blood flow interfered with by arterial occlusion, injection of adrenin and stimulation of the vagosympathetic respectively. In *D* and *E* the gland is activated by stimulation of the chorda tympani and the flow of blood interfered with by arterial occlusion and the injection of adrenin.

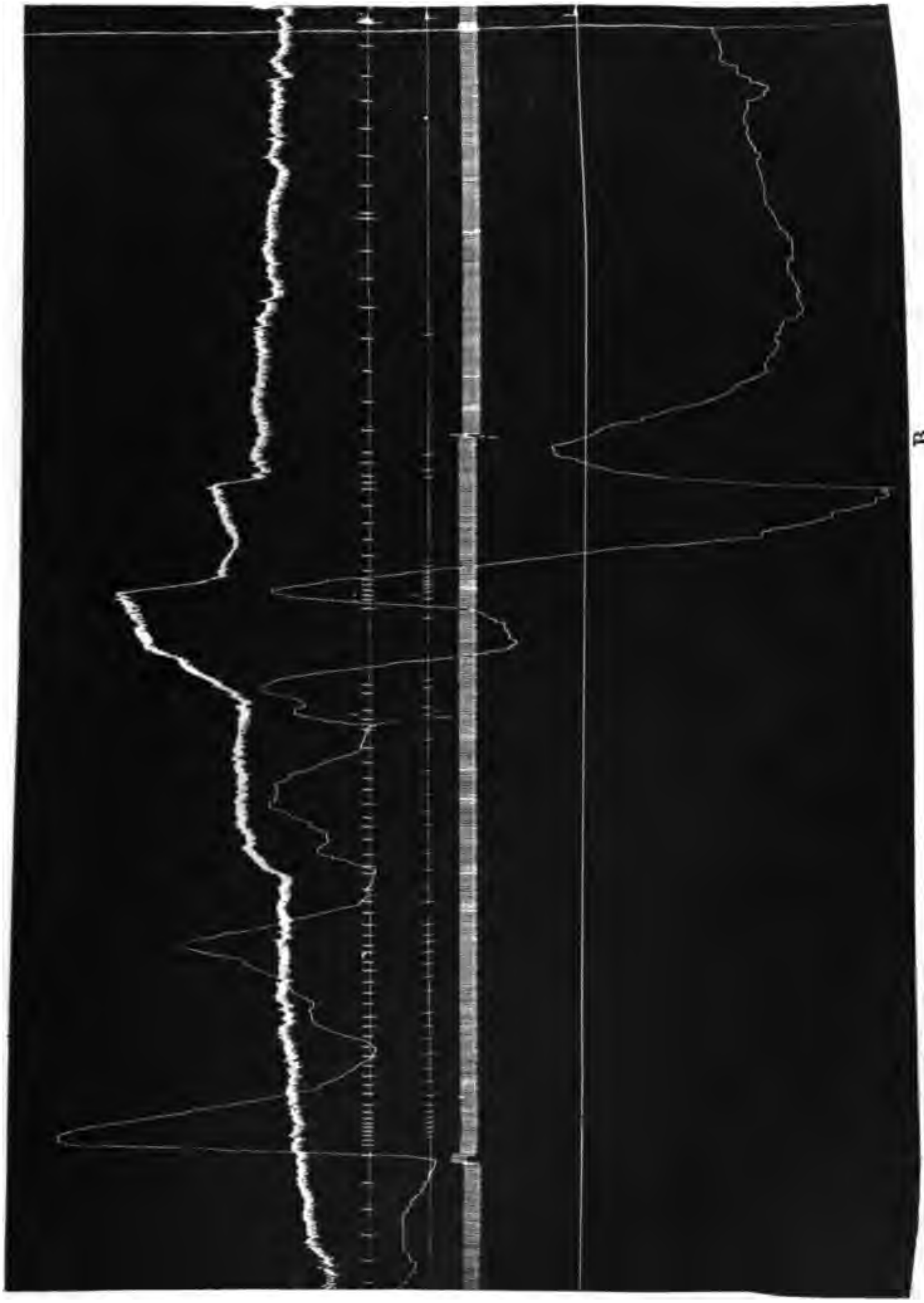

Fig. 5. Relation of secretion to volume-flow of blood.

occlusion of the carotid artery during excitation affected the processes in the gland is indicated by another change in the electrical deflection. When the artery was de-occluded about 20 seconds after the cessation of stimulation a rapid flow of blood occurred. This accelerated blood flow was accompanied by a copious secretion. The fact that an accelerated blood flow was associated with secretion some time after the effects of stimulation had wholly or at least partially worn off, seems to indicate that secretion in this instance occurred in two definite stages. The results are in line with the observation that the elicitation of visible secretion requires a stronger stimulus than does the elicitation of an electrical deflection which is an index to glandular activity. There may, however, be another explanation of the results shown in figure 6. We know from previous work that adrenin, provided it does not interfere with the flow of blood through the gland, may occasionally augment secretion elicited by either stimulation of the chorda tympani or injection of pilocarpin. It is possible that the short period of arterial occlusion led to an asphyxial discharge of adrenin into the circulation (5) sufficient to augment secretion. Unfortunately results similar to those seen in figure 6 were not obtained frequently enough to make possible the determination of the factor underlying the accelerated secretion.

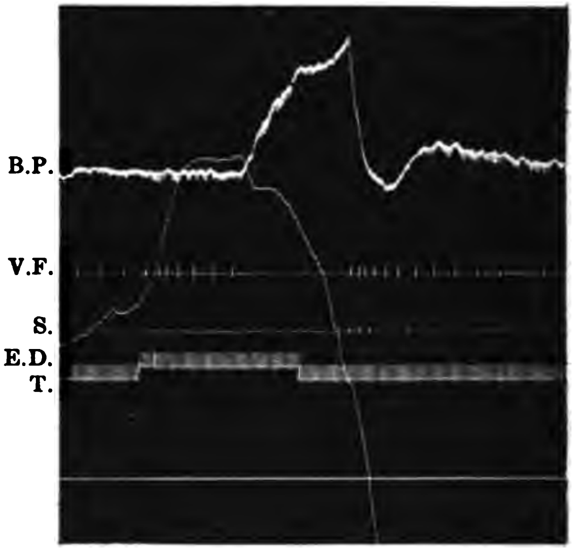

Fig. 6

THE MECHANISM CONTROLLING THE VOLUME-FLOW OF BLOOD

It is well known that after atropinization of an animal stimulation of the chorda tympani may accelerate the volume-flow of blood through the submaxillary gland without eliciting visible secretion. This observation is used as evidence of the presence of vasodilator fibers in the chorda tympani. But Barcroft (6) pointed out that even though there be no visible secretion resulting from stimulation of the chorda tympani, oxidations in the gland may be increased. The accelerated flow of blood may, therefore, be due to liberation of dilator metabolites rather than to stimulation of dilator fibers.

The fact that stimulation of the chorda tympani, too weak to produce visible secretion, may elicit an increased volume-flow of blood is likewise cited as evidence for the presence of dilator fibers in that nerve. But the fact that such an increase in volume-flow of blood is accompanied by an electrical deflection also makes possible the explanation of dilatation through dilator metabolites (1).

Since these methods, which indicated the existence of dilator fibers, fail to yield crucial data concerning the mechanism of volume-flow control, I attempted in a previous research to throw further light on the problem by a variety of indirect methods (1). The results obtained were summarized as follows:

> As to the existence of vasodilator nerves the question which initiated this research nothing definite can be said. We have no proof that such nerves do not exist, neither have we proof that metabolites can not adequately control the volume-flow of blood. All that can be said is, that if the dilator fibers do control the volume-flow of blood, this flow may be augmented still more by an accumulation of metabolites. Many observations might apply to both theories, some however point more strongly to the metabolite control.

It was shown in that research that with conditions constant the flow of blood is very finely adjusted to the activity of the gland. By plotting the superbasal flow of blood on the ordinates against progressively increasing amounts of salivary secretion on the abscissas we found superbasal flow of blood to be a linear function of superbasal metabolism. Such accelerated flow accompanying tissue activity is undoubtedly a purposive reaction to make good the excess of liberated energy, but does not help toward determining the mechanism of volume-flow control. This reaction of accelerated flow of blood is studied to advantage by plotting continuous curves of glandular activity and volume-flow elicited by stimulation of the chorda tympani. This method not only brings out in another way the parallelism of the two phenomena but in addition shows the time relation of the two processes.

Figure 7 shows the effect of stimulation of the chorda tympani of 12 seconds duration in 7, *A*, and of 60 seconds in 7, *B*. The activity of the gland (secretion) is plotted on the ordinates in solid black against time on the abscissas. Volume-flow of blood is plotted as a line. The portion of the curve of volume-flow of blood preceding stimulation of the chorda tympani represents the basal flow of blood or the flow of rest; the curve above that level represents the superbasal flow. Though in each instance the increased flow of blood followed stimulation promptly, there was considerable lag in the flow as indicated by a comparison of the crests of the curves of secretion and volume-flow of blood. This lag or after-flow of blood, which continues throughout the experiment, is suggestive of a recuperative process following activation, and is in agreement with the findings of Barcroft and Hill on oxidation and heat formation associated with tissue activation.

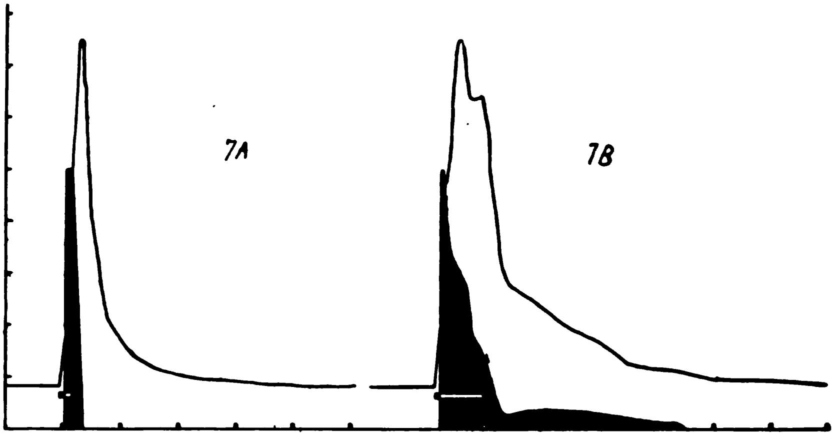

Fig. 7. Effect of stimulation of the chorda tympani on secretion and volume-flow of blood. Volume-flow of blood and salivary secretion are plotted on the ordinates against time in minutes on the abscissas. The duration of stimulation of the chorda tympani is shown.

Figure 8 shows results obtained in other experiments. The graphs were obtained from four different animals. Graphs *A* and *B* show the relation of volume-flow of blood to prolonged activity of the gland elicited by prolonged excitation of the chorda tympani. The usual effect of such stimulation upon secretion is a rapid acceleration followed by a decrease which in turn gives way to a secondary increase. The parallelism between secretion and volume-flow of blood is striking.

Graph 8, *C* and *D*, shows the same relation, but the fluctuations in secretion differ from those in graph 8, *A* and *B*, in that they are elicited by short periods of stimulation. Secretion elicited by stimulation of

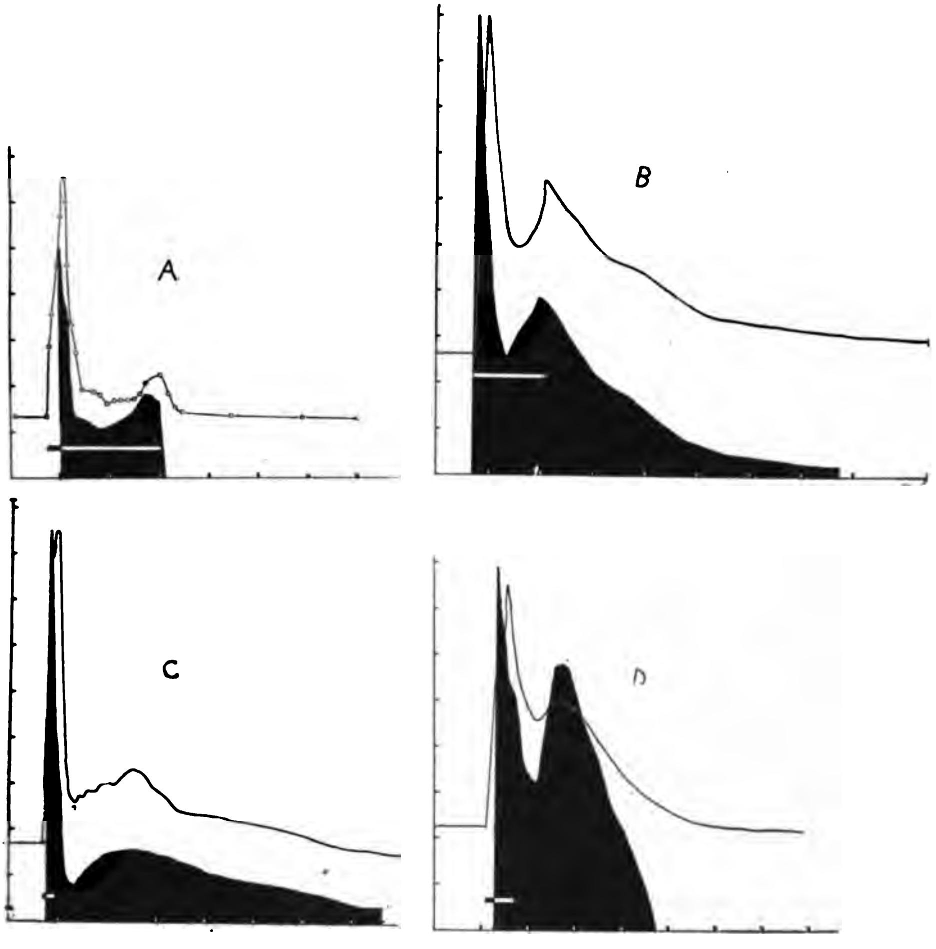

Fig. 8. Effect of stimulation of the chorda tympani on secretion and volume-flow of blood. Volume-flow of blood and salivary secretion are plotted on the ordinates against time in minutes on the abscissas. *A* and *B* show the relation of volume-flow of blood to prolonged secretion elicited by prolonged stimulation of the chorda tympani. *C* and *D* show the relation of volume-flow of blood to prolonged secretion elicited by a short period of stimulation of the chorda tympani.

the chorda tympani as a rule stops promptly upon cessation of stimulation, but not infrequently, the secretion slows only to accelerate before ultimately decreasing. Such results are represented in graph 8, *C* and *D*. The volume-flow of blood here, too, is closely adjusted to the activity of the gland. It is interesting to consider the significance of these results in relation to the mechanism of vasodilatation. The after-secretion following cessation of stimulation may last from 1 to 15 minutes. The cause of this secretion we do not know. It may possibly be due to a prolonged after-discharge of the vasomotor post-ganglionic cells. A like discharge of the vasomotor post-ganglionic cells would explain in a similar manner the after-flow of blood. If both after-flow of blood and after-flow of secretion are due to this after-discharge of ganglionic cells it is extremely interesting that the after-discharge of the two sets of cells should be so nearly equal and so exactly timed.

Numerous experiments similar to those represented in figures 7 and 8 show that under constant conditions and regardless of the duration of stimulation, the volume-flow of blood is nicely adjusted to the needs of the tissues. Although such results need not favor either theory of vasomotor control, the lag of accelerated volume-flow following activation might suggest at least the coöperation of metabolite control.

If a lack of parallelism could be demonstrated between volume-flow of blood and activity of the gland resulting from stimulation of the chorda tympani, the presence of dilator fibers in the chorda tympani would be suggested, only, however, assuming the absence of vasoconstrictor fibers. But it appears to be a difficult matter to demonstrate satisfactorily such a lack of parallelism. Perhaps graph 8, *D*, could be looked upon as representing a slight lack in the perfection of adjustment between volume-flow and tissue activity, in that the second increase of secretion is associated with a relatively smaller increase in volume-flow than that associated with the first phase of secretion. In a few experiments the lack of parallelism has been far more striking in that the volume-flow of blood actually decreased during a period of copious secretion.

As to the significance of these apparent exceptions, it is obvious that the presence of a variable number of constrictor fibers in the chorda tympani might change the relation between glandular activity and volume-flow of blood elicited by stimulation of the nerve. Fröhlich and Loewi (7), believe that such fibers exist. They obtained a decreased flow of blood, such as is described above, when the chorda tympani was stimulated. In their experiments nitrites were administered for the purpose of producing a maximum dilatation, thereby permitting effective stimulation of the constrictor fibers running in the chorda tympani. Bayliss (8) failed to obtain the decreased flow of blood under the conditions given by Fröhlich and Loewi and furthermore failed to confirm the results by stimulation of the cervical sympathetic nerve of the cat, which is known to contain constrictor fibers.

I attempted to determine the presence of constrictor fibers running in the chorda tympani by selective stimulation of these fibers produced by hemorrhage. I recorded the volume-flow of blood from both submaxillary glands during progressive hemorrhage. On one side the chorda tympani and the vago-sympathetic were cut and on the other side only the vago-sympathetic. Since one gland was connected with the central nervous system through the chorda tympani and the other gland was completely isolated, if constrictor fibers are present in the chorda tympani we might expect a difference in the curves of basal flow of the two glands (basal flow of blood plotted on the ordinates against mean blood pressure upon the abscissas). A comparison of the curves of basal flow of blood failed to indicate the presence of constrictor fibers in the chorda tympani.

The results shown in figure 9 are more helpful in explaining the difference between the results of Fröhlich and Loewi and of Bayliss. In this figure the curve of secretion and of basal-flow of blood and of salivary secretion elicited by short periods of stimulation of the chorda tympani at various levels of blood pressure during progressive hemorrhage are plotted. The period of stimulation in each case lasted about 20 seconds. The curve beginning on the abscissas is the curve of secretion. The other is the curve of blood flow. The horizontal portion preceding stimulation of the chorda tympani represents the flow of rest or basal-flow and the remaining portion the secretory or super-basal flow of blood. Record *A* was obtained during normal blood pressure. In record *D* the pressure had fallen to about 40 mm. Hg.

From the work of Barcroft we know that during secretion water is abstracted from the blood flowing through the gland. As the basal-flow of blood diminishes with decreasing pressure the basal and super-basal-flow of blood decrease and the configuration of the curves changes in a way indicative of this abstraction of water, for it will be noted that in the final stages of hemorrhage the flow of blood during secretion is actually less than the flow of rest. The irregularities of the curves of blood flow in graphs *B* and *C* apparently are the result of abstraction of water from the blood, but only when the blood pressure is too low to take sufficient advantage of the dilatation which presumably occurs, does the abstraction of water reduce the flow below that of rest. This reduction gives the appearance of constriction.

The exceptions to the proportionality of tissue activity and volume-flow of blood, therefore, can not be said to be real, but figure 10 shows results which may possibly be of significance. This figure shows the effect of reducing in steps the strength of a prolonged and continuous stimulation of the chorda tympani. The period of stimulation lasted approximately 7 minutes. Figure 10, *A*, shows the usual results of such a procedure and figure 10, *B*, the unusual results. In figure 10, *A*, it will be noted that at the points *B*, *C* and *D*, where the strength of current was decreased, volume-flow of blood and secretion showed proportional changes. Not so in figure 10, *B*. For some reason the response of the gland to the same procedure was strikingly different. The chorda tympani was stimulated at *A* and the strength of stimulation kept constant up to *B*. During this period the usual relations between

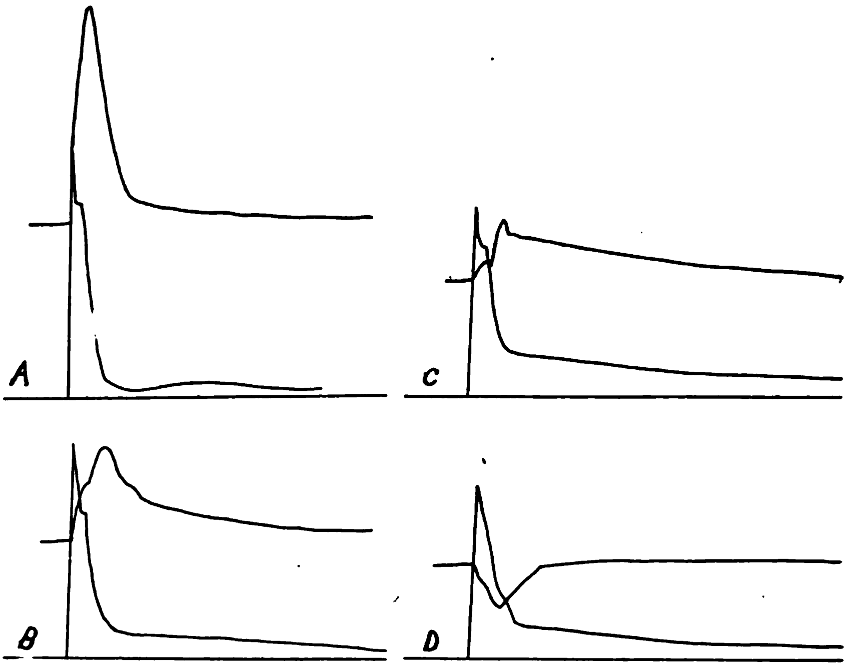

Fig. 9. Relation of volume-flow of blood to secretion during progressive hemorrhage.

volume-flow of blood and secretion obtain. At *B* the strength of stimulation was suddenly decreased and, barring the smaller variations

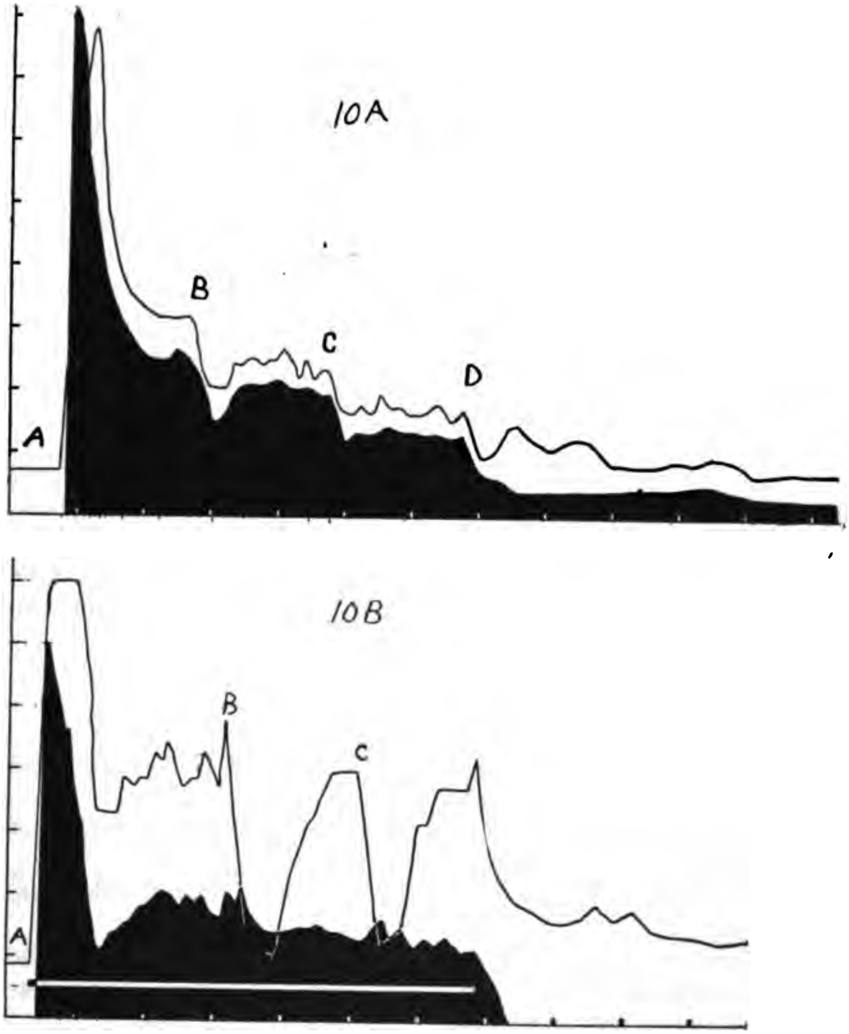

Fig. 10. Effect of suddenly reducing in steps the strength of a prolonged stimulation of the chorda tympani.

in rate of secretion, there was little if any change of rate. On the other hand the volume-flow of blood was enormously reduced—almost to the basal flow of blood. Though the strength of stimulation and rate of secretion remained constant up to point *C*, the flow of blood remained reduced for a short period only, coming back again to the original superbasal flow. Another sudden reduction in the strength of stimulation at point *C* produced the same results.

It is exceedingly interesting that the reduced volume-flow of blood should suddenly accelerate though the strength of stimulation remained constant between the points of sudden diminution of strength of stimulation, to reach again the level obtaining between *A* and *B* where the stimulation is considerably stronger. From the fact that the volume-flow of blood diminished though the secretion remained constant, it would appear, assuming the presence of dilator fibers, that the strength of stimulation was reduced below the threshold of these fibers. Whether the subsequent acceleration of volume-flow of blood is due to an automatic lowering of the threshold of stimulation of the dilator fibers resulting from accumulation of metabolites or whether it is due to the direct action of the metabolities on the vessels, the results do not definitely indicate. They do show a lack of parallelism between metabolism and volume-flow and accepting the absence of constrictor fibers in the chorda tympani they point to the existence of dilator fibers. The value to be placed on these findings depends upon the significance we can attach to an isolated exception of this kind. It should be mentioned in this connection that although this result was obtained on only one animal it was obtained repeatedly.

SUMMARY

The dependence of tissue activity on volume-flow of blood was studied on the submaxillary gland of the dog—

a, by comparing the amount of secretion obtained during periods of normal and reduced flow of blood;

b, by noting the effect of decreased flow of blood upon the electrical deflections;

c, by studying the after-flow of blood following de-occlusion of the artery immediately following tissue activation. (An exaggerated after-flow of blood was used as an index to overstrain of the tissue).

Reduction of the volume-flow of blood during a short period of stimulation of the chorda tympani, from 10 to 30 seconds, did not decrease the amount of secretion.

The glandular processes, however, were affected by such procedure, for the electrical deflections were invariably altered.

Reduction of the volume-flow of blood during a period of more intense stimulation, but also of short duration, although it did not reduce the amount of secretion elicited, resulted in a more prolonged flow of blood as well as an altered electrical deflection.

With more prolonged stimulation of the chorda tympani the various glands responded differently to arterial occlusion. On some glands occlusion of the artery for a period of about 1 minute was without effect upon secretion, while in others a noticeable reduction in secretion occurred.

The temporary independence of tissue activity of volume-flow of blood as evidenced by secretion is probably apparent only and is due to the recovery of the gland between periods of stimulation and to the relative independence of the process of liberation of secretion on volume-flow of blood.

The dependence of tissue activity upon flow of blood is better shown by reducing the flow through a tissue which has already been activated for some minutes, that is, in a tissue in which recuperation and activity are going on hand in hand.

Such methods showed a very close dependence of tissue activity upon volume-flow of blood. The results substantiate the views previously published on the significance of hemorrhage and reduced flow of blood from other causes in the onset and sustentation of the condition of traumatic shock.

Prolonged stimulation of the chorda tympani usually produced fluctuations in secretion during the period of stimulation: first a rapid secretion followed by a decrease, then another acceleration giving way to a final decrease at the end of stimulation. Similar fluctuations were produced by a short period of stimulation lasting only a small part of the period of secretion. Whether the stimulation was long or short, the volume-flow of blood and the secretion ran parallel with each other. The significance of the findings is discussed.

The close parallelism between tissue activity and volume-flow of blood offered difficulties in demonstrating definitely the existence of dilator fibers.

One experiment showing a lack of this parallelism indicates the presence of dilator fibers in the chorda tympani.

Data are presented indicative of chemical regulation of blood flow.

Experiments are cited pointing to the absence of constrictor fibers in the chorda tympani.

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STUDIES ON THE SUBMAXILLARY GLAND

VII. ON THE EFFECTS OF INCREASED SALIVARY PRESSURE ON THE ELECTRICAL DEFLECTIONS, THE VOLUME-FLOW OF BLOOD AND THE SECRETION OF THE SUBMAXILLARY GLAND OF THE DOG

ROBERT GESELL

From the Departments of Physiology of Washington University Medical School and the University of California

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In studying the electrical deflections of the submaxillary gland of the dog I noted that stimulation of the chorda tympani produced a greater and more prolonged after-flow of blood when the salivary duct was obstructed than when unobstructed (see figs. 4, 5 and 8). In so far as these observations aid in elucidating the mechanism of the control of volume-flow of blood they pertain to the problem of papers II, III, IV, VI and VIII of this series. The observations, however, will be considered from a broader point of view, namely, in their relation to the general problem of the physiology of the salivary glands.

The data will be discussed under the following heads:

1. Effects of increased salivary pressure upon the electrical deflections of the gland.
   - *a.* Occlusion of the salivary duct during secretion elicited by the injection of pilocarpin.
   - *b.* Occlusion of the duct synchronous with secretion elicited by the stimulation of the chorda tympani.
   - *c.* Backward injection into the salivary duct of the resting gland.
2. Effects of increased salivary pressure upon the volume-flow of blood through the gland.
   - *a.* Occlusion of the salivary duct during secretion elicited by the injection of pilocarpin.
   - *b.* Occlusion of the duct synchronous with secretion elicited by the stimulation of the chorda tympani.
   - *c.* Backward injection into the salivary duct of the resting gland.
3. Effect of increased salivary pressure upon secretion.
   - *a.* Occlusion of the duct during secretion elicited by the injection of pilocarpin.
   - *b.* Occlusion of the duct synchronous with secretion elicited by stimulation of the chorda tympani.

c. Backward injection into the salivary duct of the resting gland.

d. Effect of occlusion of the duct during secretion elicited by the stimulation of the chorda tympani upon secretion elicited by subsequent stimulation of the chorda tympani and vago-sympathetic.

e. Effect of backward injection in the resting gland upon subsequent secretion elicited by stimulation of the chorda tympani or vago-sympathetic before and after atropinization.

1. EFFECTS OF INCREASED SALIVARY PRESSURE UPON THE ELECTRICAL DEFLECTIONS OF THE GLAND

a. Occlusion of the duct during secretion elicited by the injection of pilocarpin. When the submaxillary gland is activated by the injection of pilocarpin a definite electrical deflection occurs and when the secretion approaches a constant rate the two electrodes tend again to assume a constant difference of potential as is evidenced by the horizontal direction of the recorded electrical deflection. If the salivary duct is now occluded a typical disturbance of this equilibrium occurs which is shown in figure 1, *A*, *B*, *C*, *D* and *E*. The first effect of occlusion was an upward deflection which gave way in a few seconds to a downward deflection. De-occlusion produced the reverse effect. The downward deflection accompanying occlusion was suddenly accelerated and changed as suddenly into an upward deflection. The contour of the deflection differed considerably from time to time and from animal to animal as is obvious from figure 1, *A*, *B*, *C*, *D* and *E*, yet the four phases were present in all. The electrical deflection elicited by occlusion and de-occlusion of the duct may therefore be looked upon as more or less accurately indicating the sequence of certain glandular processes set up by these procedures.

b. Occlusion of the salivary duct synchronous with secretion elicited by the stimulation of the chorda tympani. If the chorda tympani is stimulated at regular intervals with stimuli of equal strength and duration equal amounts of saliva may be elicited and electrical deflections of the same contour may be obtained, provided, the period of rest intervening between stimulations is of such duration as to prevent the augmenting effect of previous excitation. When such constant results were obtained it was found that occlusion of the salivary duct along with stimulation of the chorda tympani produced a definite change in the electrical deflection as is seen in figures 2 and 3. These figures show five sets of observations—2, *A*, *B* and *C*, and 3, *A* and *B*. In any single set of observations the chorda tympani was stimulated at equal intervals of time with equal strength of stimulation and the duct occluded equal lengths of time. But in the different sets of observations the periods and strength of stimulation, the periods of rest and of occlusion of the duct were variable. This variability must in part account for the differences of the deflections. Bearing the variability of the conditions of the experiments in mind it is quite remarkable that the changes in contour produced by occlusion of the duct should be as uniform as they are.

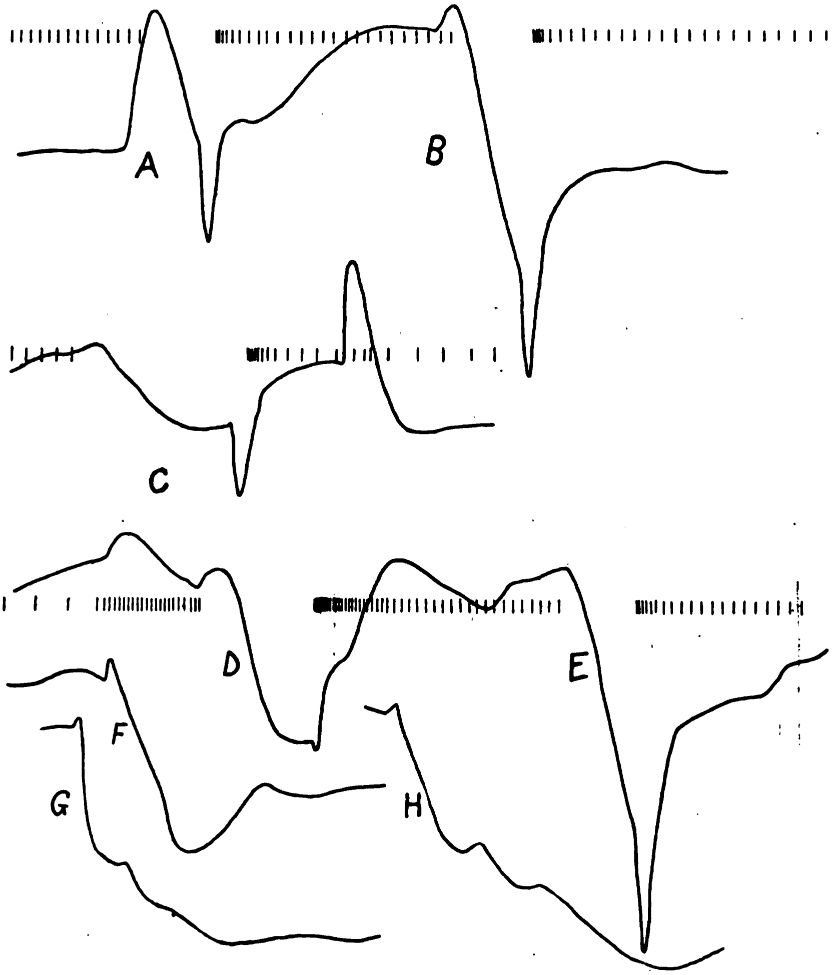

Fig. 1

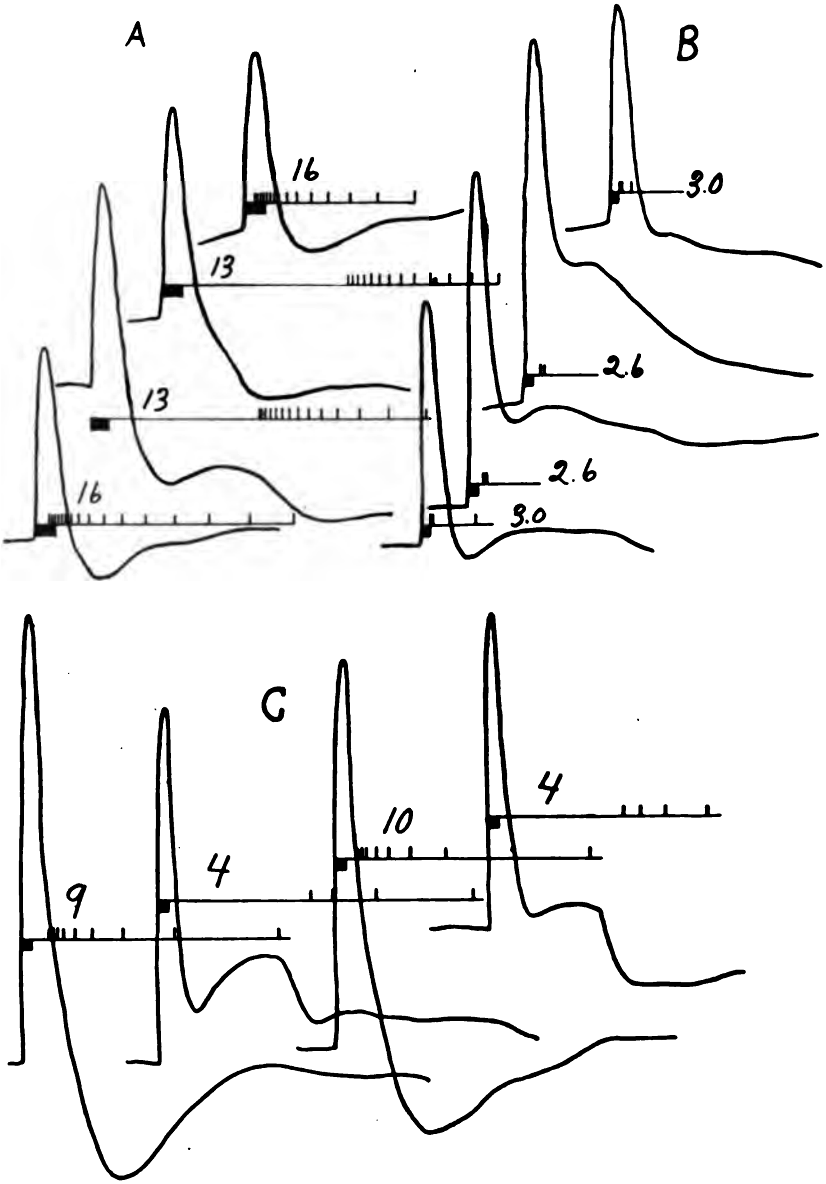

Fig. 2

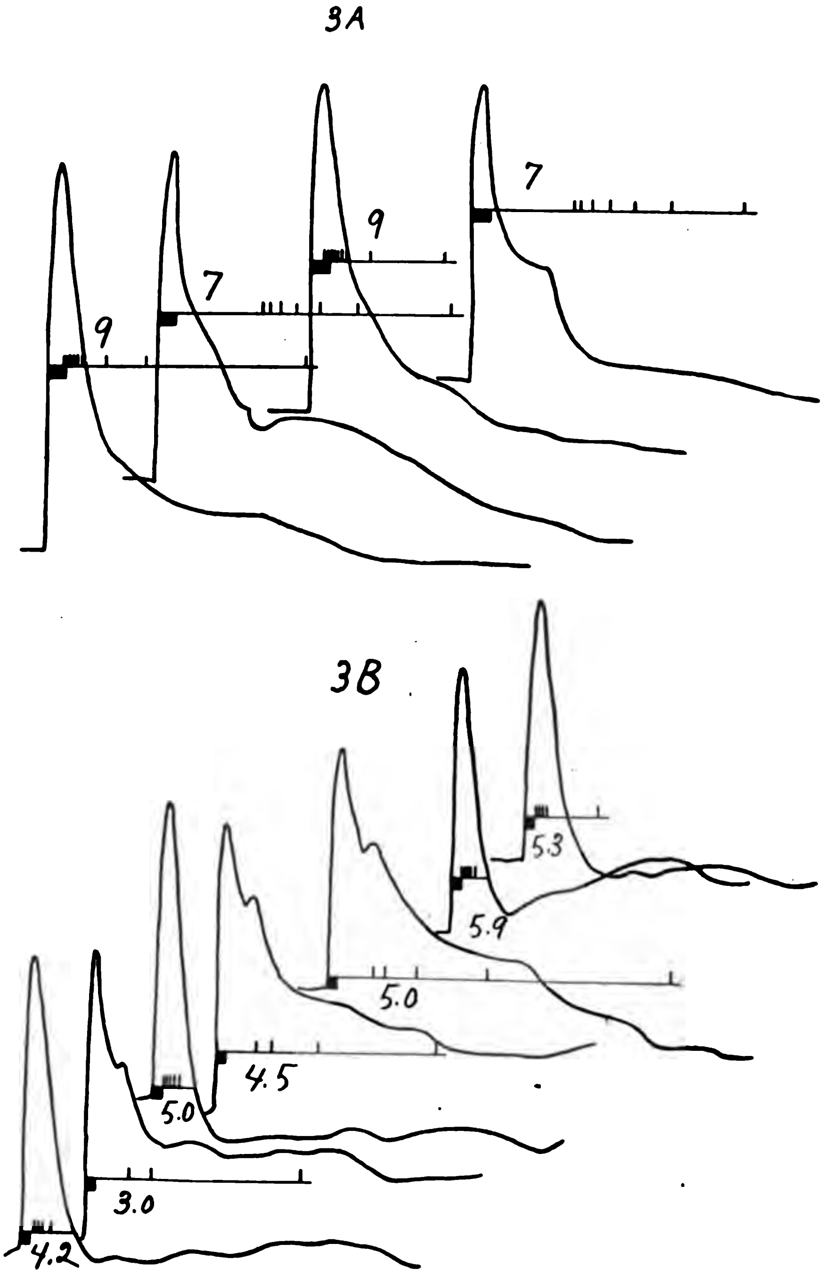

Fig. 3

Prolongation of the electrical disturbance is a point more or less common to the observations in which occlusion of the duct occurred. This prolongation is associated with a prolongation of the normal period of secretion. Other characteristics common to many of the modified deflections are the notch and the elevation of the base line. To be sure there are some dissimilarities in the deflections of different groups of observations, yet in any single set of observations repeated occlusion produced changes in the deflections so nearly alike that here again the deflections seem to indicate with considerable exactness the sequence of glandular processes.

In some instances de-occlusion of the duct produced a downward deflection similar to that associated with de-occlusion during secretion from the injection of pilocarpin. But it is obvious that the deflection as a whole showing the effects of occlusion and de-occlusion cannot be compared to advantage with the deflections obtained by occlusion and de-occlusion during secretion from the injection of pilocarpin, for in one instance the deflection is a result of a change in secretion already in progress and in the other it is a resultant of the activation of the resting gland and the obstruction of the secretion formed.

c. *Backward injection into the salivary duct of the resting gland.* The backward injection of saline or gum-saline produced deflections comparable to those elicited by occlusion of the duct during active secretion elicited by the injection of pilocarpin. Such deflections are shown in figure 1, *F*, *G* and *H*. The injection produced an upward deflection followed by a downward deflection. Cessation of the backward injection was associated with a downward deflection followed by an upward deflection. The general contour of the deflection, to be sure, differs from that produced by occlusion during active secretion, yet the four phases are present. It is of interest to note here that atropinization apparently does not influence the effects of backward injection upon electrical deflections.

2. EFFECTS OF INCREASED SALIVARY PRESSURE UPON THE VOLUME-FLOW OF BLOOD THROUGH THE GLAND

a. *Occlusion of the salivary duct during secretion elicited by the injection of pilocarpin.* Occlusion of the salivary duct during secretion resulting from the injection of pilocarpin as a rule retarded the flow of blood during the period of occlusion, as is shown in figure 5, *A*. On de-occlusion the flow accelerated again to reach or surpass the flow preceding occlusion. The de-occlusion flow of blood in figure 5, *A*, only approximated the pre-occlusion flow, but in consideration of the fact that the volume-flow of blood as a rule is proportional to the activity of the gland and of the fact that the de-occlusion secretion was considerably slower than the pre-occlusion secretion, the results suggest that even in this observation occlusion in reality produced an accelerated after-flow of blood.

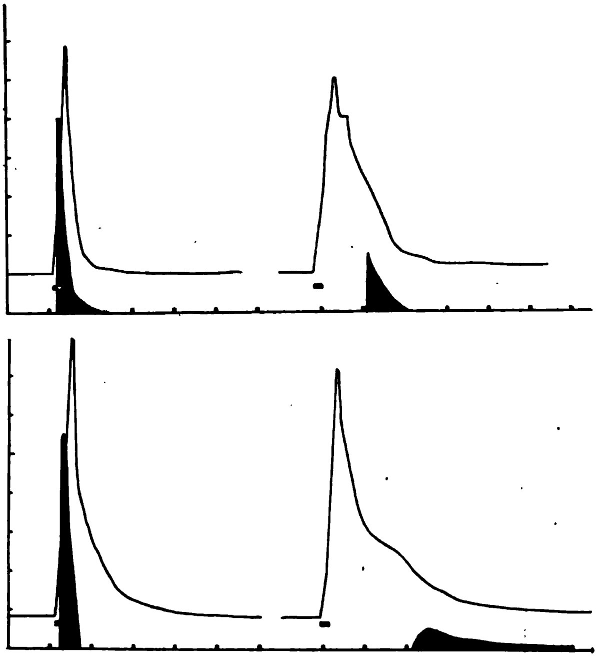

Fig. 4

b. Occlusion of the duct synchronous with secretion elicited by stimulation of the chorda tympani. The above results are substantiated by the universally accelerated after-flow of blood noted on de-occlusion of the duct after occlusion synchronous with secretion elicited by stimulation of the chorda tympani (see figs. 4 and 6). A comparison of record *B* of figure 6, in which occlusion of the duct occurs, with records *A* and *C*, in which there was no occlusion of the duct, shows the accelerated flow of blood. The results of two such experiments are plotted in figure 4 in which secretion (solid black) and volume-flow of blood (single line) are plotted on the ordinates against time in minutes

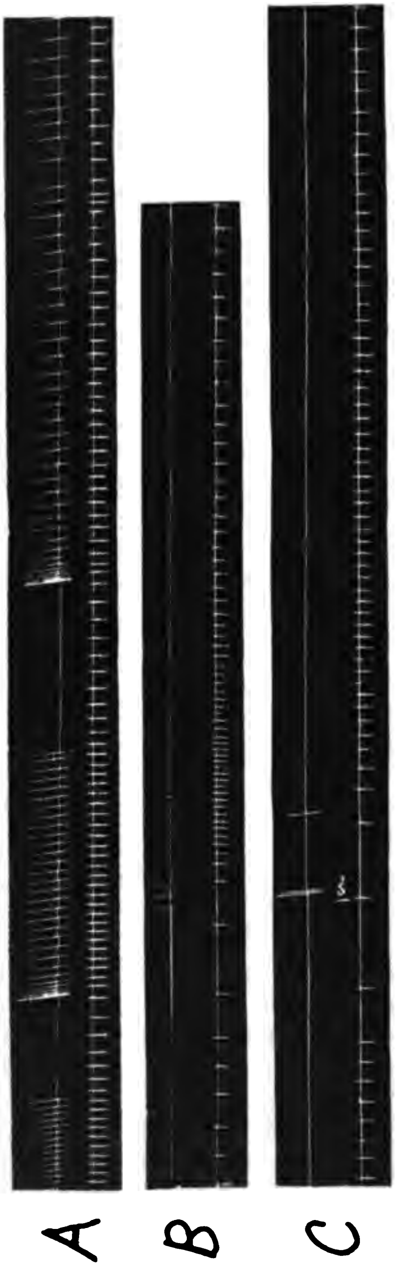

Fig. 5

on the abscissas. The duration of stimulation of the chorda tympani is marked by the rectangle near the abscissas at the beginning of the record. The extra-flow of blood elicited when the duct was occluded greatly exceeded the extra-flow occurring when the duct was not occluded. Though the two experiments represented in this figure show little reduction of the flow of blood during the occlusion of the duct, such slowing not infrequently occurred, in agreement with the results produced by occlusion of the duct during secretion elicited by the injection of pilocarpin.

c. Backward injection into the salivary duct of the resting gland. Backward injection into the salivary duct of the resting gland produced comparable results to those obtained by increasing the salivary pressure in the two ways discussed above (see fig. 5, *B* and *C*). During the period of increased salivary pressure the flow of blood was greatly reduced. On cessation of injection, which is indicated by the salivary record, the volume-flow of blood accelerated to remain accelerated for several minutes above the basal-flow of blood preceding injection.

3. EFFECT OF INCREASED SALIVARY PRESSURE UPON SECRETION

a. Occlusion of the duct during secretion elicited by the injection of pilocarpin. De-occlusion of the salivary duct after occlusion during secretion elicited by the injection of pilocarpin was followed by a momentary rapid acceleration of secretion which within a few seconds usually gave way to a rate of secretion below that obtaining before occlusion (see fig. 5, *A*). How much of the momentary accelerated secretion was due to the emptying of distended ducts, to the passage of saliva which escaped into the tissue spaces back into the ducts, to the liberation of secretion accumulated in the cells themselves due to failure to overcome the increased salivary pressure in the ducts, the experiments do not show. The retarded secretion following de-occlusion suggests some form of tissue damage resulting, possibly, from backward filtration. The effect which occlusion of the duct has upon the rate and amount of secretion following de-occlusion depends largely upon the duration of occlusion, the rate of the pre-occlusion secretion and the variability common to the glands themselves. Some saliva is always unaccounted for in the compensatory secretion.

b. Occlusion of the duct synchronous with secretion elicited by stimulation of the chorda tympani. Occlusion of the salivary duct during stimulation of the chorda tympani followed by subsequent de-occlusion produced variable results, depending again upon the rate of secretion elicited by stimulation, the duration of occlusion of the duct and the peculiar reaction of the gland itself. Various results from several animals are shown in figures 2 and 3. The records in each case are arranged in their proper sequence. Figure 2, *A* and *B*, which is compiled from two different animals, shows the effects of prolonged occlusion of a copious secretion and a momentary occlusion of a scant secretion. In figure 2, *A* the chorda tympani was stimulated at regular intervals of 4 minutes with a constant strength of stimulation lasting 16 seconds. With the duct unobstructed each stimulation elicited 16 drops of secretion as is shown in the first record of that series. In the following two records where the duct was occluded for 2.5 minutes 13 drops of saliva were secreted following de-occlusion, that is, only 3 drops of secretion were lost. In the final control when the duct was unoccluded 16 drops were again elicited. In the following record, 2, *B*, a scant secretion of only 3 drops of secretion was elicited by a relatively weak stimulus lasting 5 seconds. The duct was occluded only 10 seconds. It will be noted that occlusion of such scant secretion for only 10 seconds resulting in a loss of about 13 per cent of the secretion and producing a definite change in the electrical deflection, stands in striking contrast to the loss of only 3 drops out of a larger total of 16 drops of secretion obstructed for 2.5 minutes. The results in figure 2, *B* indicate that the storage capacity of the ducts of a gland weighing approximately 7 gm. may be little over 2 drops and that if an amount greater than 2 drops is obstructed, enough back pressure may be developed to interfere with further secretion. If that is true, where were the 13 drops of the experiment represented in 2, *A* stored?

c. Backward injection into the salivary duct of the resting gland. An index to the capacity of the ducts as it pertains to this problem may be indicated by the after-flow of secretion following backward injection of gum-saline in the resting gland. When a small amount of fluid was injected and then retained by occlusion of the duct, subsequent de-occlusion within 40 to 80 seconds was usually followed by several drops of after-flow. Not infrequently there was no after-flow whatever; but the usual flow of 2 to 4 drops suggests again that the ducts may accommodate, for a short time at least, approximately 3 drops of saliva.

d. Effect of occlusion of the duct during secretion elicited by the stimulation of the chorda tympani upon secretion elicited by subsequent stimulation of the chorda tympani and the vago sympathetic. The effects of occlusion of the duct during secretion elicited by the stimulation of the chorda tympani upon secretion elicited by subsequent stimulation of the chorda tympani and vago sympathetic are shown in figure 6. This figure gives the results of two sets of observations, *A-D* and *W-Z*. The record of the first set shows secretion and volume-flow of blood; the record of the second set electrical deflections as well. The chorda tympani was stimulated at regular intervals with stimuli of equal strength and duration. Equal amounts of secretion were elicited with each stimulation under normal conditions. During one such period of stimulation the salivary duct was occluded and the effect upon the subsequent stimulation noted. In record *A* a total of 12 drops of saliva was secreted. Since 3 of these drops were secreted slowly after the cessation of stimulation, only 7 drops were rapidly secreted during the period of stimulation. In record *B* the duct was occluded during stimulation of the chorda tympani and several minutes after stimulation the duct was de-occluded whereupon only one drop of saliva fell from the cannula. Next, record *C*, the chorda tympani was stimulated with the duct de-occluded; 13 drops of saliva were secreted, of which 11 drops appeared within the 10-second interval of stimulation. In record *D*, the final control, 6 drops of saliva were secreted rapidly. It follows from these results that occlusion of the duct during secretion increased the subsequent secretion by 4 or 5 drops.

The augmenting effect of occlusion upon secretion is shown still better in observations *W*, *X*, *Y* and *Z*, due to the fact that after-secretion was absent. In record *W* stimulation of the chorda tympani for a period of 10 seconds elicited 4 drops of saliva. In record *X* the duct was occluded during stimulation. A rapid flow of blood occurred. There was no flow of saliva on de-occlusion of the duct. In the following record, *Y*, stimulation elicited 8 drops of saliva or double the amount in record *W*. In the final control approximately 3 drops were again secreted. Note the electrical deflections.

The effects of occlusion of the salivary duct during secretion elicited by stimulation of the chorda tympani on subsequent secretion elicited by stimulation of the vago sympathetic appear to be identical with those just described (see fig. 7). The lower broken line on which time is marked in seconds indicates when the chorda tympani was stimulated and the upper line when the vago-sympathetic was stimulated. The results of alternate stimulation of the chorda tympani and vago-sympathetic without occlusion of the duct are shown in the upper tracing; with occlusion, in the lower tracings. Without previous occlusion of

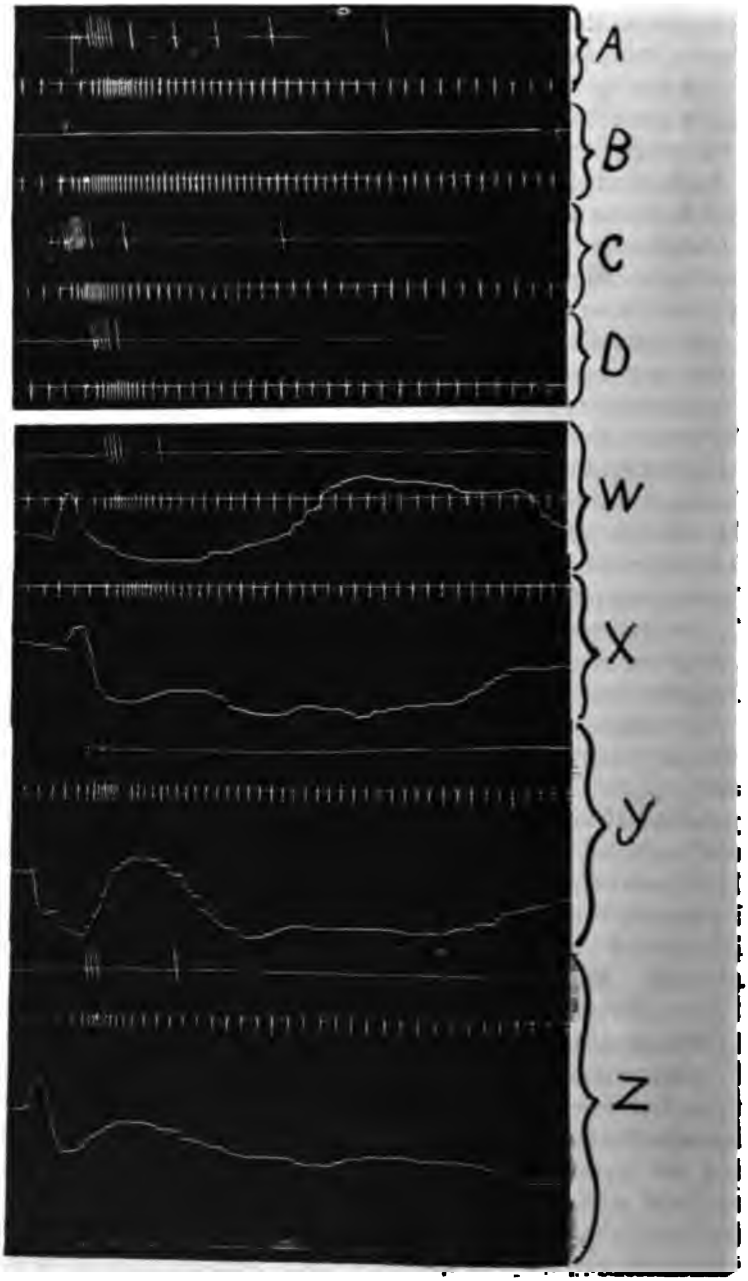

Fig. 6

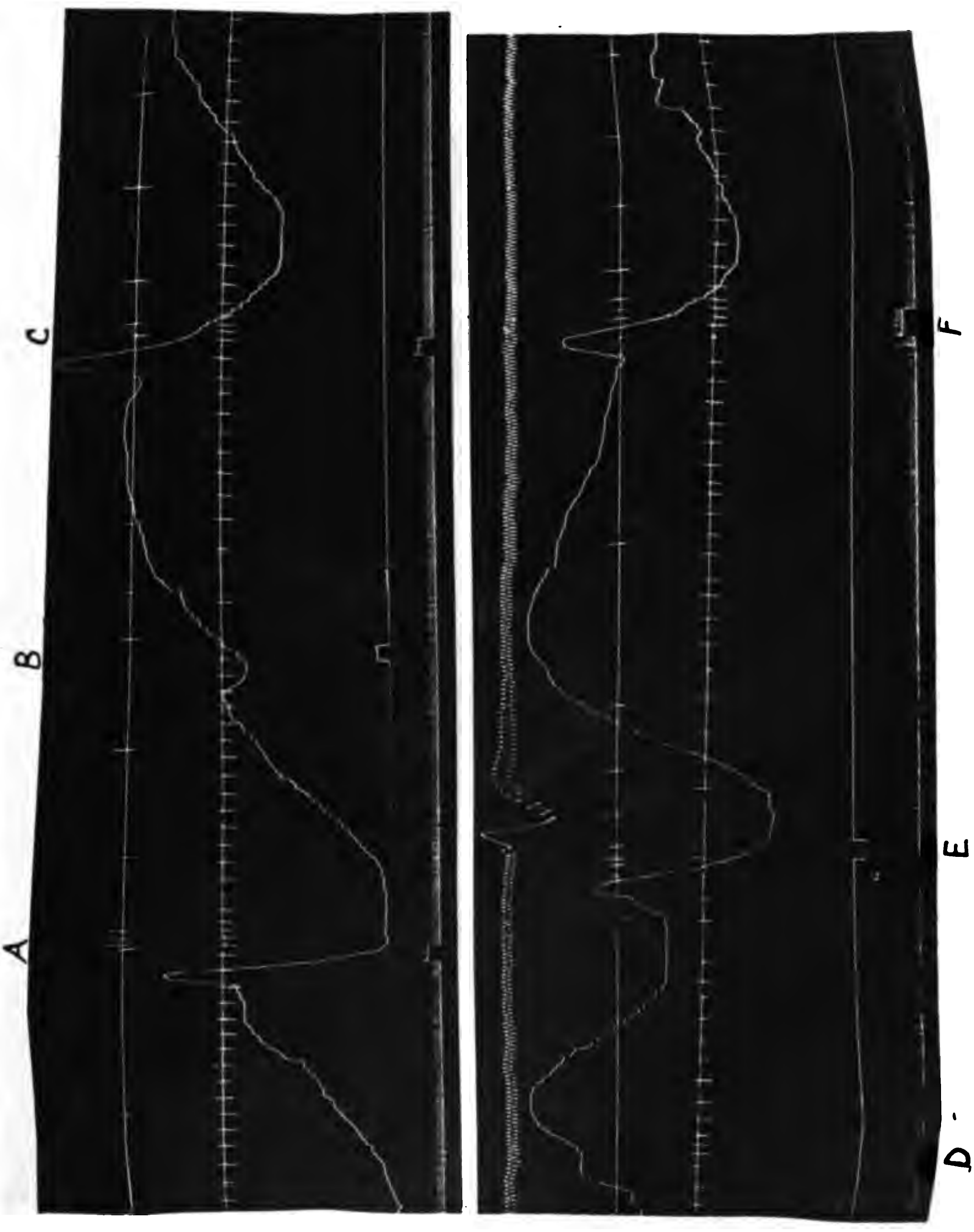

Fig. 7

the duct stimulation of the vago-sympathetic elicited a slow scanty secretion of only 3 drops, but with previous occlusion a secretion of 8 drops.

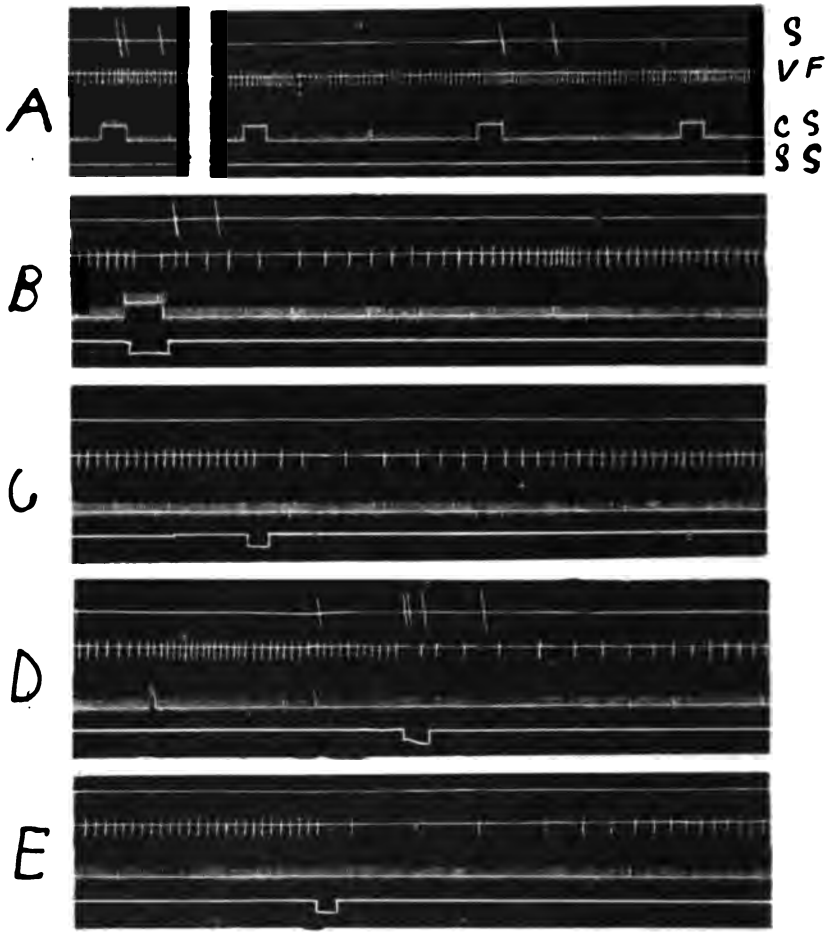

Fig. 8

e. Effect of backward injection in the resting gland upon subsequent secretion elicited by the stimulation of the chorda tympani or vago-sympathetic before and after atropinization. When equal amounts of saliva were elicited at regular intervals by equal stimulation and gum-saline was then momentarily injected into the duct, it was found that stimulation of the chorda tympani 1 to 5 minutes following the injection elicited a greater amount of secretion and that the latent period of secretion was reduced by several seconds. The same reduction in latent period was noted when stimulation of the chorda tympani was preceded by occlusion of the duct during secretion elicited by stimulation of the chorda tympani.

The experiment represented in figure 8 shows the effect of atropin upon the results described above. In record *A*, the first stimulation of the chorda tympani elicited 3 drops of saliva. Following that stimulation 1 mgm. of atropin was injected intravenously. This effectively paralyzed the chorda tympani for the prevailing stimulus, as is shown by the absence of secretion with the second stimulation. Gum-saline was then injected backwards through the duct into the gland and the duct de-occluded within a minute. The next stimulation elicited 2 drops of secretion though the subsequent stimulation was ineffective. In record *B*, a continuation of the experiment, the chorda tympani and vago-sympathetic were simultaneously stimulated producing 2 drops of secretion. The accelerated volume-flow of blood shortly following was a result of the injection of 11 mgm. of atropin. Stimulation of the vago-sympathetic following that injection in record *C* was ineffective so far as secretion is concerned, but reduced the flow of blood. In record *D*, gum-saline was injected into the duct between the breaks on the time record. One drop left the cannula on de-occlusion. Subsequent stimulation of the vago-sympathetic yielded 4 drops and final stimulation none. Similar results have been obtained after the intravenous injection of 350 mgm. of atropin.

DISCUSSION

From the work of Langley we know that during rest some of the constituents of salivary secretion are elaborated and stored ready for subsequent secretion. It appears that even after the glandular cells are well stocked with zymogen granules, secretion may yet occur in two definite stages, final elaboration followed by ultimate liberation. Various observations point in that direction, e.g., the observation of Langley on the augmenting effect of stimulation of the chorda tympani upon the secretion elicited by subsequent stimulation of the sympathetic fibers. Stimulation of the chorda tympani too weak to elicit secretion may produce a definite electrical deflection. In the preceding paper it was noted that a gland subjected to a subnormal flow of blood failed to secrete during the period of stimulation of the chorda tympani, whereas it secreted 20 to 30 seconds after cessation of stimulation when the flow of blood through the gland was suddenly accelerated. The observation in the present paper, that a gland may continue to secrete for a period of 4 or 5 minutes after the cessation of stimulation, as is shown in the de-occlusion experiments, is likewise of significance; as is also the fact that injection of atropin in amounts sufficient under usual experimental conditions to paralyze the secretory endings of the chorda tympani, fails to prevent a discharge from the gland on stimulation of the chorda tympani after a previous injection of gum-saline into the duct of the gland.

Unfortunately we do not understand the reaction of the gland to increased salivary pressure. The fact that a storage of more than 2 drops of saliva in the ducts embarrasses the gland and that 13 drops of saliva may be secreted after the duct is occluded for a period of 2.5 minutes indicates that at least not all of the after-secretion following de-occlusion is due to an expression of saliva contained within the distended ducts. The slowness of the secretion would also speak against this. Yet we have some evidence pointing to the contractility of the salivary ducts; for example, when the gland is secreting at an even rate as a result of an injection of pilocarpin, a short stimulation of the chorda tympani results in a momentary acceleration of secretion followed by a compensatory slowing which in turn gives way to a rate approaching the initial rate.

We know that when secretion occurs against a high resistance much of the saliva is filtered backward into the interspaces of the gland. The extreme slowness of secretion in certain instances following de-occlusion of the duct suggests the passage of this saliva back into the ducts. So far as I know we have no evidence that an increased salivary pressure results in filtration of saliva back into the secreting cells themselves or prevents the liberation of saliva which is already in these cells. Since "secretion" may be elicited following backward injection after atropinization sufficient to paralyze secretion, there is the possibility that under more normal conditions fluid is filtered backward into the cells, this fluid being later liberated. A part of the excess fluid within the cells may be liberated by a passive mechanism on the reduction of the salivary pressure accompanying de-occlusion, but the passive mechanism may not be sufficient to liberate all of the excess fluid or secretion and this may then be actively liberated upon stimulation of the chorda tympani. It seems not improbable that this active liberation may be the result of active contraction of certain constituents of the secreting cells themselves. This problem needs further investigation and will be reported upon later. The results so far indicate only that atropin in certain stages of atropinization paralyzes primarily the function of elaboration of secretion leaving the function of liberation more or less intact.

As to the nature of the changes in volume-flow of blood produced by increased salivary pressure we can say that they are not of a central reflex origin if all the vasomotor nerves to the gland run in the chorda tympani and vago-sympathetic, for these changes in flow occurred after section of both nerves. The decreased flow obtaining during the period of increased pressure may well be a mechanical effect of compression of the capillaries and venules, for it obtains during stimulation of the chorda tympani when the factors normally producing dilatation are at work, as well as when the gland is at rest. On de-occlusion of the duct the compressing pressure vanishes and the flow of blood not only returns to normal but greatly exceeds the normal. When this accelerated flow was first noticed with occlusion accompanying stimulation of the chorda tympani it was thought that possibly more energy was required for secretion against a high pressure and that the accelerated flow of blood supplied this needed energy. The accelerated flow resulting from backward injection into the duct of the resting gland is contradictory to this view. The accelerated flow does not seem to be due to a specific chemical irritation of the saliva itself for it occurs after injection of inert solution, such as saline and gum-saline. Whether a backward filtration into the secreting cells would bring about increased volume flow of blood we do not know. The only suggestion we have at present to account for the accelerated flow is the tissue damage resulting from backward filtration into the tissue spaces and possibly into the cells themselves.

SUMMARY

Occlusion of the duct of the submaxillary gland of the dog during secretion elicited by the injection of pilocarpin produced characteristic electrical deflections.

Injection of gum-saline into the duct produced somewhat comparable deflections.

Occlusion of the duct along with stimulation of the chorda tympani modified in a more or less typical way the usual electrical deflection obtained by stimulation when the duct was not occluded.

Occlusion of the salivary duct during secretion elicited by the injection of pilocarpin retarded the flow of blood. The after-flow of blood was at times accelerated.

Backward injection of gum-saline into the salivary duct of the resting gland retarded the flow of blood during the period of increased pressure. Release of that pressure resulted in an accelerated flow of blood at times lasting many minutes.

Occlusion of the duct during secretion elicited by stimulation of the chorda tympani frequently retarded the flow of blood. De-occlusion was followed by a markedly accelerated after-flow of blood.

De-occlusion of the salivary duct during secretion elicited by the injection of pilocarpin was followed by a short period of accelerated secretion. This accelerated secretion never compensated fully the absence of secretion during the period of occlusion.

De-occlusion of the duct several minutes subsequent to injection of gum-saline into the duct was followed by an after-flow from the duct of 0 to 4 drops.

Occlusion of the salivary duct along with stimulation of the chorda tympani was followed upon de-occlusion by a secretion less in amount than that normally elicited with the duct unoccluded. The amount of saliva unaccounted for varied considerably with the animal, the duration of occlusion and the amount of secretion obstructed.

De-occlusion of the duct after obstructing for several minutes a temporary secretion elicited by stimulation of the chorda tympani may be followed by a slow secretion lasting 4 to 5 minutes.

Occlusion of the duct during secretion elicited by the stimulation of the chorda tympani increased the amount of saliva elicited by subsequent stimulation of the chorda tympani or vago-sympathetic. The latent period of secretion was also reduced by several seconds.

Backward injection of gum-saline into the resting gland increased the amount of secretion elicited by subsequent stimulation of the chorda tympani or vago-sympathetic and reduced the latent period of secretion.

Injection of atropin sufficient to check secretion elicited by the stimulation of the chorda tympani or vago-sympathetic may fail to prevent secretion with similar stimulation after backward injection into the salivary duct.

If all the nerve fibers reaching the submaxillary gland run in the chorda lingual and vago-sympathetic the changes in volume-flow of blood resulting from increased salivary pressure are not of a central reflex origin.

The decreased volume-flow of blood during the period of increased salivary pressure is probably due to mechanical occlusion of the capillaries or venules.

It is suggested that the accelerated flow of blood following de-occlusion is a result of tissue damage from backward filtration into the tissue spaces, and possibly into the cells themselves.

The effects of occlusion of the duct upon secretion elicited by stimulation of the chorda tympani suggests that secretion may occur in two definite stages. The effects of atropin on augmented secretion following backward injection of gum-saline into the duct suggests the same inference.

STUDIES ON THE SUBMAXILLARY GLAND

VIII. On the Effects of Atropin upon Volume-flow of Blood, Electrical Deflections and Oxidations of the Submaxillary Gland

ROBERT GESELL

From the Departments of Physiology of the Washington University Medical School, St. Louis, and the University of California, Berkeley, California

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When the chorda tympani is stimulated definite effects are produced in the submaxillary gland among which are: secretion of saliva, an augmented flow of blood, a change in the electrical condition of the gland and an accelerated oxidation. This paper considers briefly some of the effects of the intravenous injection of atropin upon these processes.

The work is a continuation of the study of electrical deflections of the submaxillary gland and has a bearing upon the problems of vasomotor control. In paper II of this series (1) the nature of the electrical deflections elicited by stimulation of the chorda tympani was discussed. It was pointed out that such factors as the type of lead, the strength and duration of stimulation, the period of rest, etc., all influence the kind of deflection obtained. It is essential then to keep these factors in mind in connection with the effects which atropin produces upon electrical deflections elicited by stimulation of the chorda tympani.

We know from the work of others the effect which atropin has upon secretion of saliva. These effects, when noted in this work will, therefore, be discussed only in their connection with the changes in volume-flow of blood, the electrical condition of the gland and oxidations.

Atropin influences the electrical response of the gland profoundly as was shown by Bayliss and Bradford (2). I have obtained like results.

Figure 1 shows the effect of the injection of 1.5 mgm. of atropin. Record *A*, shows the effects of stimulation of the chorda tympani with both vago-sympathetics intact before the injection of atropin. Record *D*, shows the effects of stimulation after double vago-section and atropinization. Records *B* and *C*, show the effects of section of the left and right vago-sympathetics respectively. Atropinization abolished

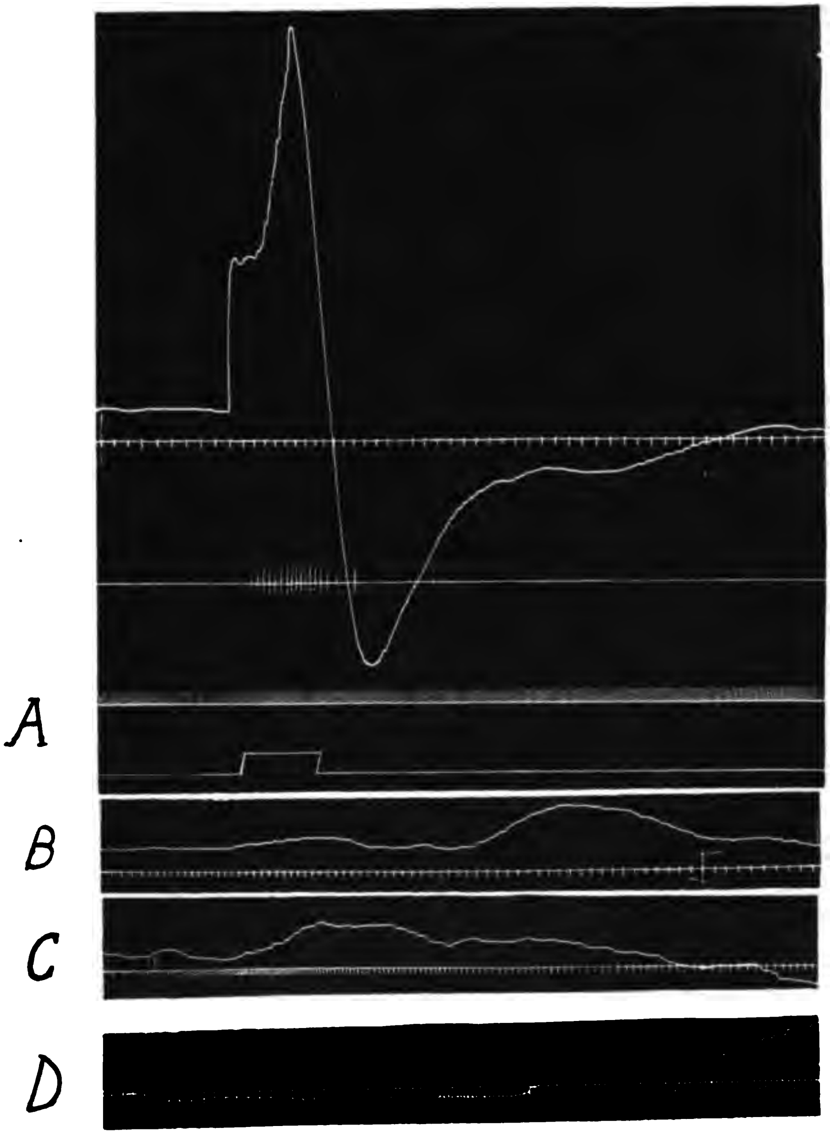

Fig. 1

both the secretion and the electrical deflection elicited by stimulation of the chorda tympani, and from records *B* and *C*, it is apparent that

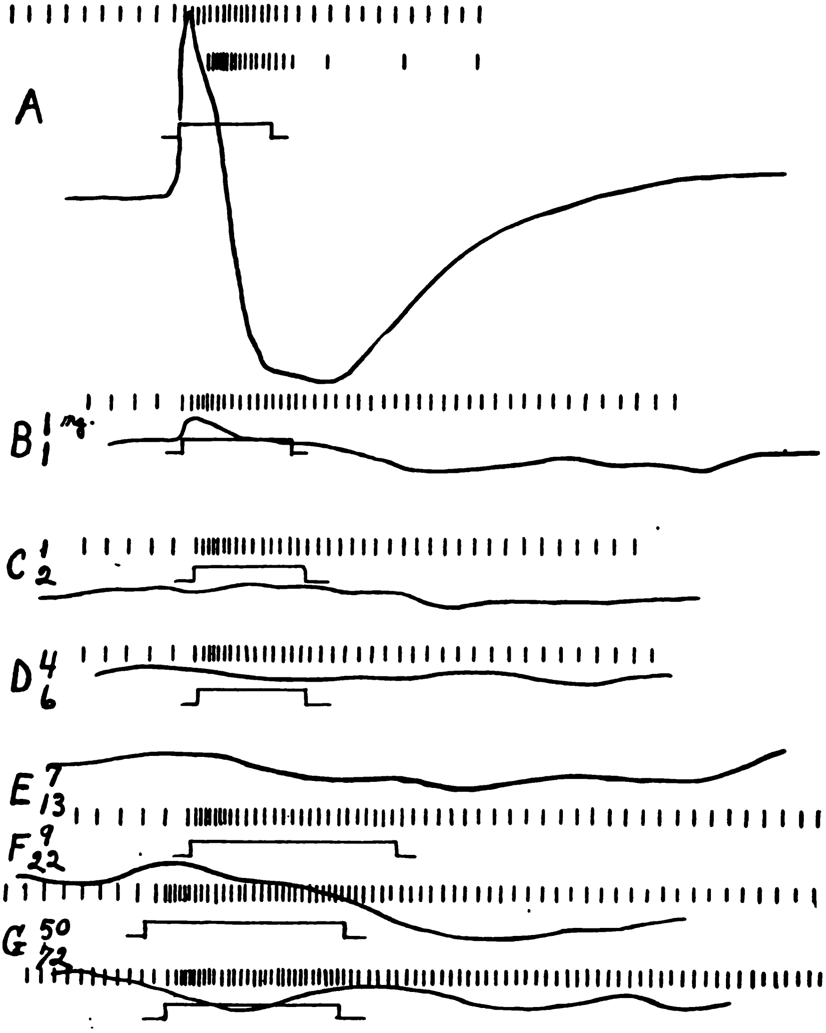

Fig. 2

volume-flow of blood per se was of little if any significance in the development of electrical changes. In record *A*, where the volume-flow of blood was least affected the largest electrical deflection occurred, and in records *C* and *D*, where the flow was enormously accelerated, both passively and actively, there was practically no change in the electrical deflection.

Figure 2 shows the effects of repeated injections of small amounts of atropin, each injection occurring several minutes before stimulation of the chorda tympani. The amounts injected are noted on the record—the upper figure represents the amount injected prior to stimulation and the lower figure the total amount injected at that moment. In record *A*, before the injection of any atropin, stimulation of the chorda tympani elicited a copious secretion, a copious flow of blood and a large electrical deflection. In record *B*, after the injection of 1 mgm. of atropin, similar stimulation elicited approximately the same flow of blood but the secretion was reduced to only ½ drop and the electrical deflection was nearly abolished. The injection of another milligram of atropin before record *C*, although exerting no further visible effect upon the volume-flow of blood, paralyzed secretion and abolished the electrical deflection. The subsequent records followed the injections of 4, 7, 9 and 50 mgm. of atropin respectively. The electrical response of the gland continued to be absent, at least the deflections which occurred during the period of stimulation of the chorda tympani were no greater than those which occurred during the periods of rest. At times the accelerated flow of blood resulting from stimulation of the chorda tympani was reduced by the injection of atropin as is apparent from a careful comparison of records *A* and *B* of figure 3. It is of interest to note in connection with figure 2 that although the accelerated superbasal flow of blood was slightly reduced, the after-flow was greatly prolonged so that the sum total of the effects was a greater superbasal flow for equal stimulation after the administration of atropin. Note that in the lower records the basal flow of blood is considerably accelerated. The acceleration was not due to a rise of blood pressure. It occurred after section of the chorda tympani and the vago-sympathetic and therefore was peripheral in origin.

Figures 3, 4 and 5 show results of other experiments. In figure 3, the administration of 0.08 mgm. of atropin nearly paralyzed secretion for the prevailing stimulus, only 1 drop was elicited as compared with 6 drops before atropinization. It likewise reduced the superbasal flow of blood. The electrical deflection was still very distinct. In figure 4, taken from a different animal, a similar injection abolished secretion entirely. The electrical deflection was still prominent and the superbasal flow of blood during the period of stimulation was hardly affected,

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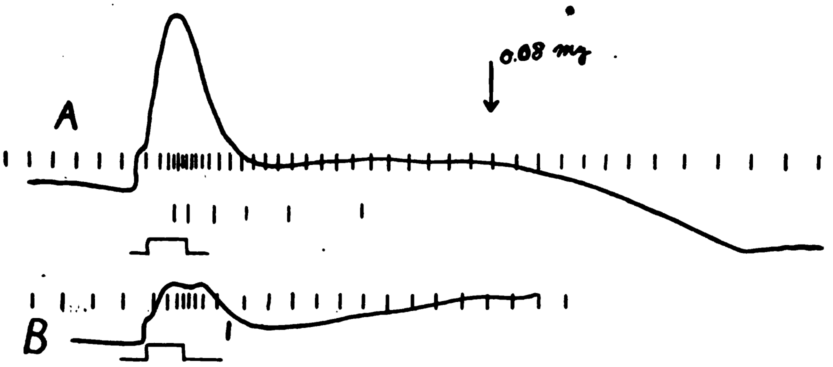

Fig. 3

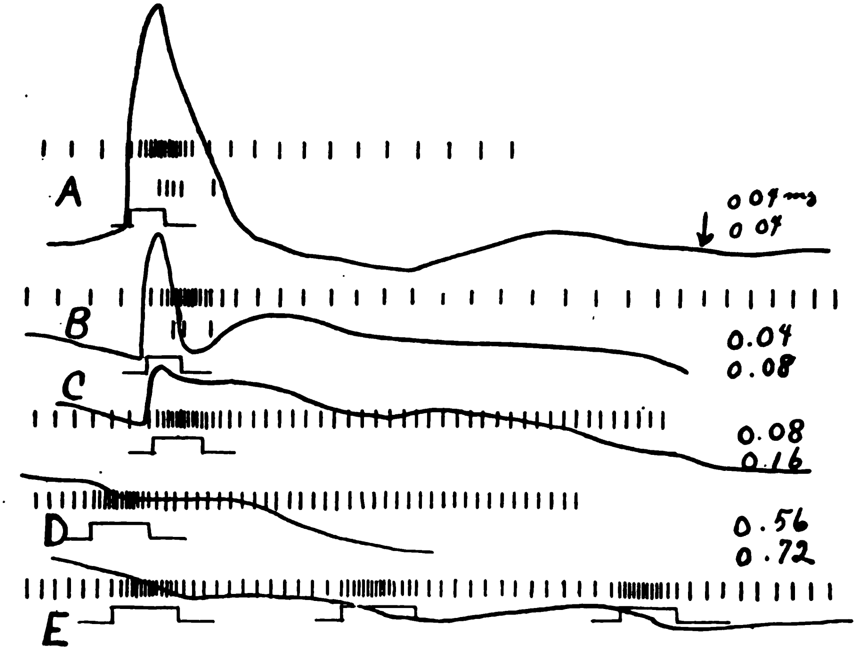

Fig. 4

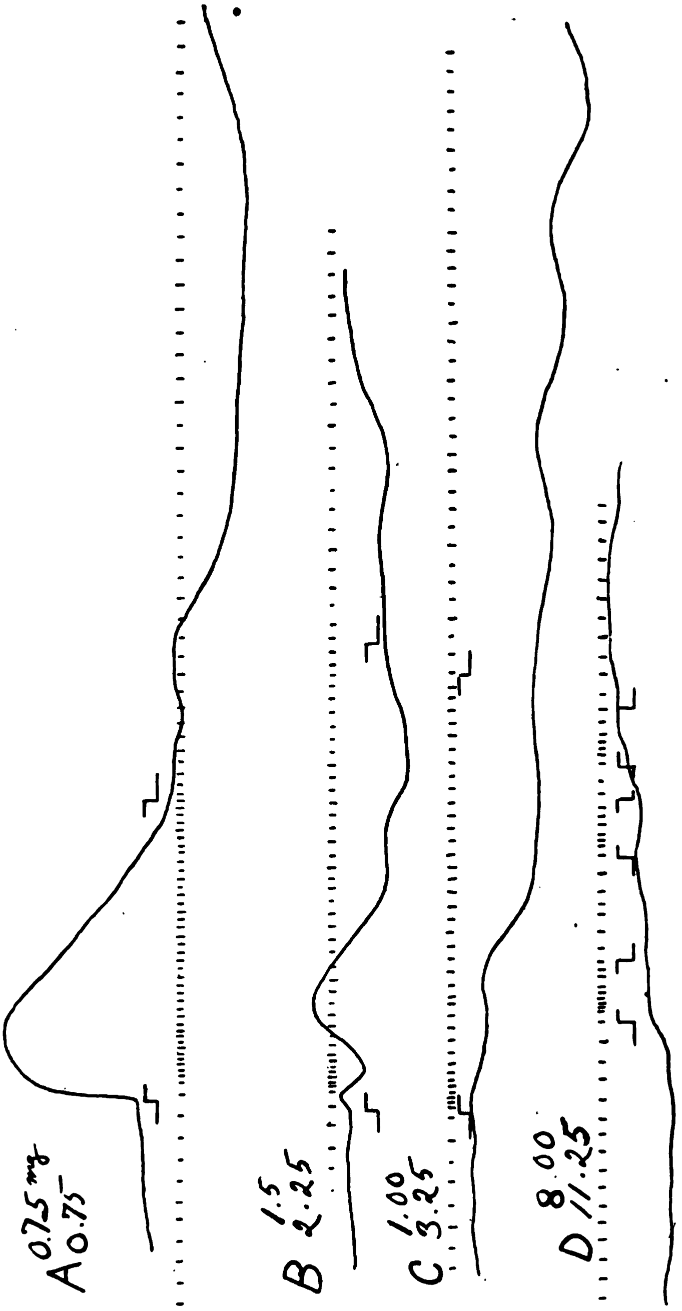

Fig. 5

but the prolongation of the superbasal flow of blood occurred again. Further injections abolished the upward deflection, but it will be noted that stimulation of the chorda tympani then produced a very small downward deflection. Though atropin usually abolished the electrical deflection this reversal was not an uncommon occurrence; but as a rule it was of small magnitude and occurred even after large injections of 30 or more milligrams of atropin.

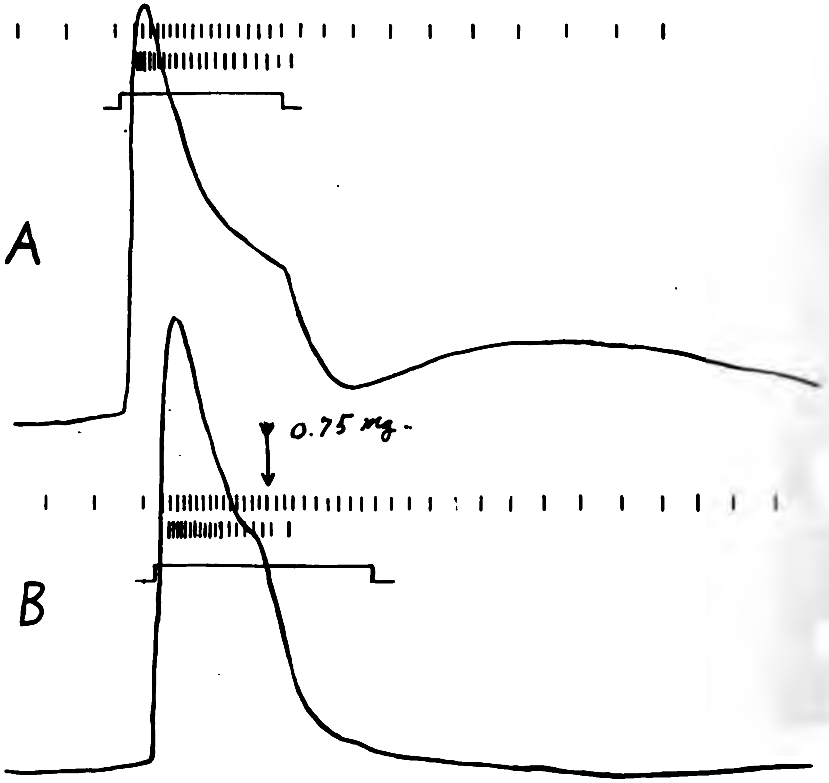

Fig. 6

If the injection of atropin prevents the development of an electrical deflection when the chorda tympani is stimulated, it might be logical to expect the injection of atropin to abolish an electrical deflection which is already in progress as a result of a continuous stimulation of the chorda tympani. Figures 6 and 7 show the results obtained on this point. In record *A*, figure 6, the chorda was stimulated before atropini-

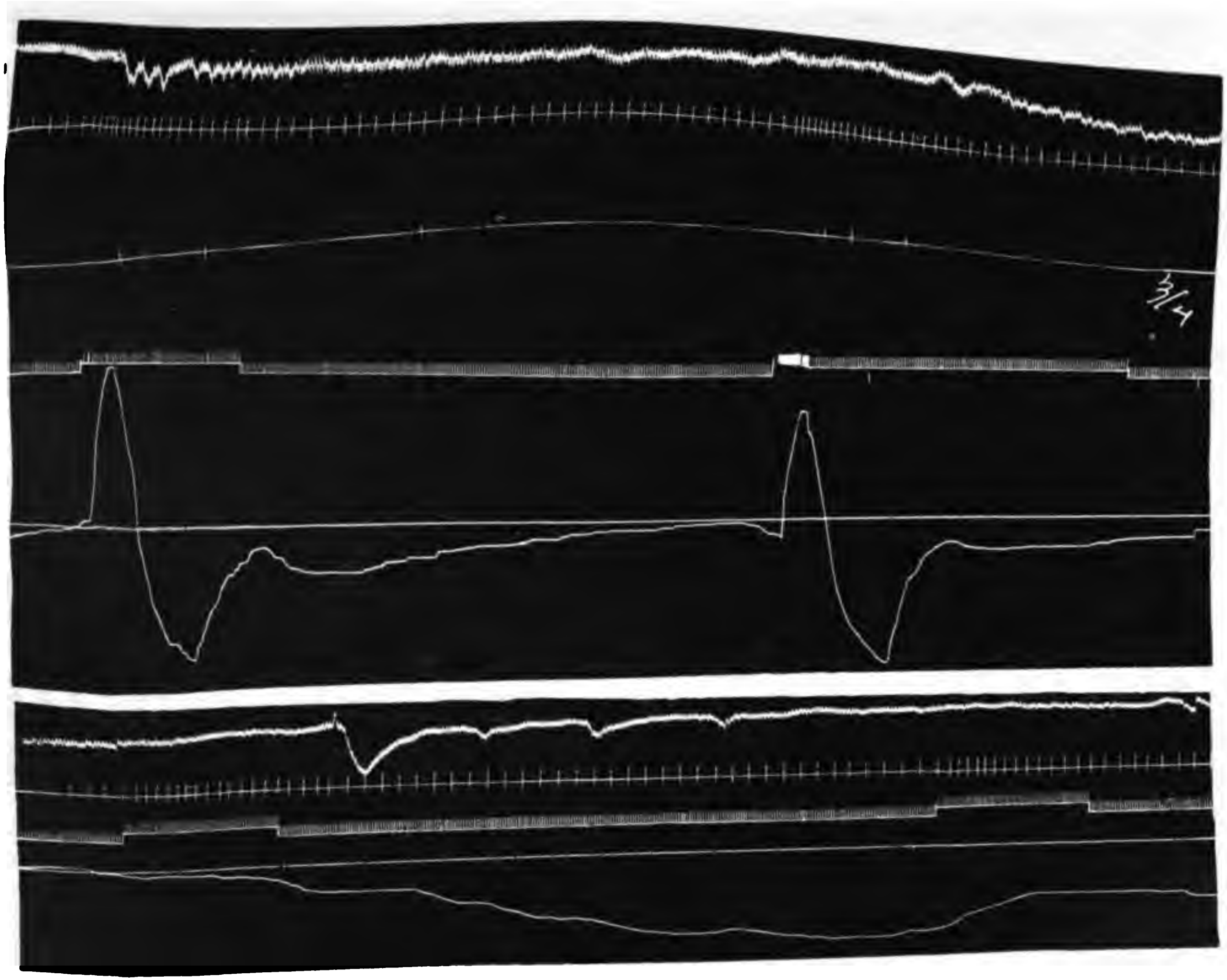

Fig. 7

zation. At the cessation of stimulation a sharp downward deflection occurred. In record *B*, atropin was injected during the period of stimulation. When the atropin reached the gland the same sharp downward deflection occurred, although the stimulation of the chorda tympani continued. In figure 7, record *B*, where the atropin reached the gland at relatively the same time interval as cessation of stimulation in record *A*, the electrical deflections were nearly identical. From the electrical deflections one might conclude, neglecting the volume-flow of blood, that atropinization during stimulation of the chorda tympani and cessation of stimulation call forth the same effects.

It is obvious that we know too little about electrical phenomena in living tissues to arrive at such a definite conclusion, yet I have tried in an indirect way to put this conclusion to the test. It should be possible to grade the strength of stimulation, or perhaps more correctly stated, the end effect of stimulation, in two ways—mechanically by regulating the strength of shock delivered by the induction coil, and physiologically by reducing the effectiveness of stimulation of constant strength by the injection of graded doses of atropin. A comparison of the electrical, secretory and vasomotor response of the gland with these two methods of gradation of stimulation seemed worth while attempting. The results obtained on two animals are shown in figures 8 and 9. The chorda tympani was stimulated at regular intervals with stimuli of equal duration. In the first observation in both experiments the stimulation was too weak to elicit visible secretion. The strength of stimulation was then increased with each observation up to an arbitrary maximum. That maximum was then kept constant for the remaining series of observations, but prior to each stimulation small amounts of atropin were injected, as small as 0.05 mgm. Each increase in strength of stimulation elicited an increase in the amount of secretion and a decided change in the electrical deflection. After the maximum strength of stimulation was reached each injection reduced the amount of secretion and elicited just as decided changes in the electrical deflection. The records show the results obtained and hardly need a lengthy discussion. To be sure, the corresponding deflections obtained with increasing and decreasing activation as indicated by the secretion are not superimposable, yet if we carefully compare the tracings of figure 8 keeping three factors in mind—the magnitude of the upward deflection, the reversal of the deflection to a downward deflection, and roughly, the general contour—definite similarities in the deflections of the two series appear. Considering the fact that identical deflections can be obtained only when many factors remain perfectly constant and that two series of deflections—one obtained with increasing strength of electrical stimulation and the other with decreasing strength of electrical stimulation—show dissimilarities, as demonstrated before, it is significant that the two series of deflections as obtained in this research have as many points in common as they do.

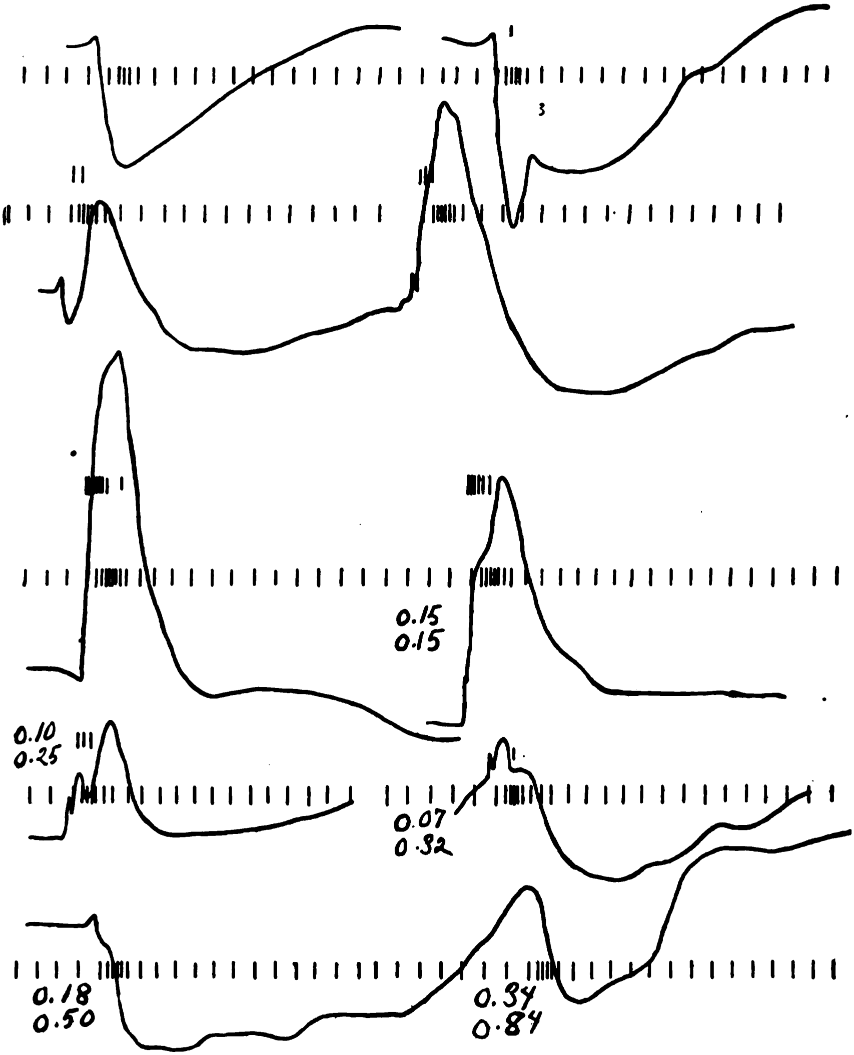

Fig. 8

If the electrical method detects minute changes in metabolic activity we should be forced to conclude that a small injection of atropin may abolish visible secretion as normally elicited by stimulation of the chorda tympani, and yet permit activation of the gland as is indicated by the marked electrical deflection which may occur in the absence of visible secretion. This conclusion agrees with the later work of Barcroft (4) on oxidations in the submaxillary gland after atropinization and is in agreement with his theory of metabolite control of volume-flow of blood. Another conclusion we should be forced to draw is that larger injections of atropin may abolish all metabolic effects which the chorda tympani has upon the submaxillary glands. This conclusion agrees with the earlier results of Barcroft (3) on oxidations in the submaxillary gland after atropinization. If correct, it reduces the impor-

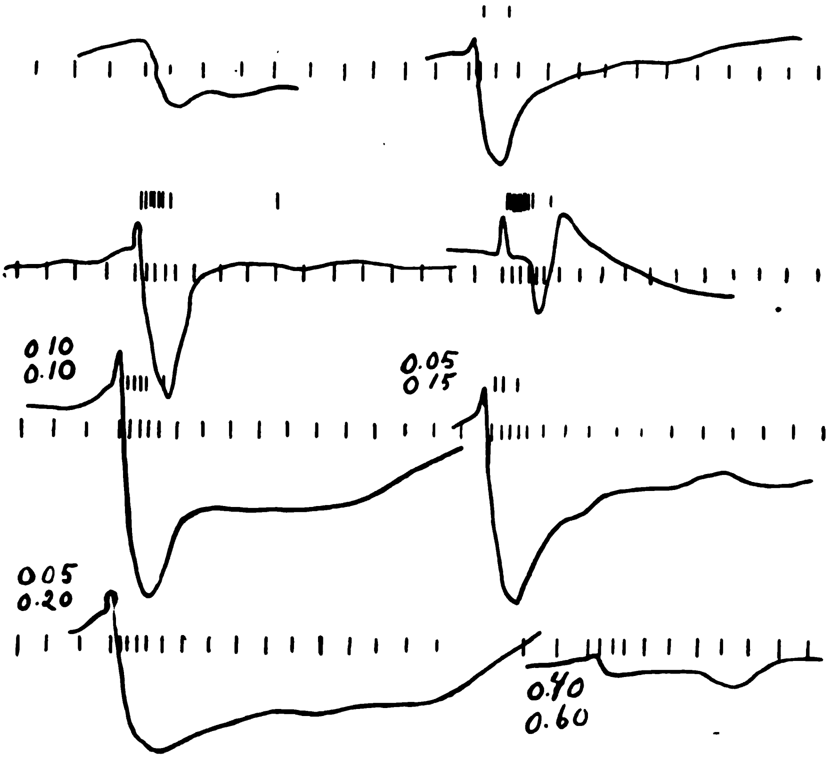

Fig. 9

tance of metabolite control, this control then only complementing the control by the vasomotor nerves.

In some experiments in which I have measured the oxidations of the gland as affected by stimulation of the chorda tympani after the injection of 3 to 5 mgm. of atropin (5), I found that although occasionally oxidations were increased as much as 15 per cent above the oxidations during rest, as a rule, oxidations were not increased by stimulation of the chorda tympani. (The Van Slyke method was used.) This amount of atropin usually abolished the electrical deflections. Oxidations were not studied after smaller injections. It would appear that the amount of atropin administered might markedly influence the results.

We know that living tissues, such as the secreting submaxillary gland, the thyroid gland, the kidney, and a nonglandular tissue such as muscle, exhibit electrical changes when their supply of blood is markedly interfered with. The inference might be that the electrical deflection is due to disturbed oxidation. In keeping with this inference is the observation that the metabolism of injured tissue is higher than that of normal tissue; on this basis the current of injury has been attributed to greater oxidations at the point of injury.

The delicacy of the electrical method of detecting metabolic activity has been demonstrated in many ways by Waller (6) in his researches on the *Signs of Life*. Yet it must be pointed out that a lack of change in electrical condition of a tissue need not necessarily indicate an absence of change of metabolic rate, for we know that a symmetrical structure such as the web of the frog's foot does not give an electrical deflection when excited symmetrically, whereas the single layer of skin of the back of the frog gives a large deflection. The magnitude of the deflection does not always vary in direct proportion to the rate of salivary secretion; in fact, occasionally the reverse happens. Apparently a balanced action of two effects of stimulation comes into play. There is, however, no evidence that symmetry of structure or perfectly balanced effects come into play more after atropinization than before.

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(4) **Barcroft**: Journ. Physiol., 1901, xxvii, 31.
(5) **Gesell**: Not published.
(6) **Waller**: The signs of life, New York, 1903.

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THE DISTRIBUTION AND QUANTITATIVE ACTION OF THE VAGI AS DETERMINED BY THE ELECTRICAL CHANGES ARISING IN THE HEART UPON VAGUS STIMULATION

E. W. H. CRUICKSHANK

From the Physiological Department of Washington University, St. Louis

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In a survey of the literature upon the vagus nerves, one can not but be struck by the fact that so many workers in this field have paid so little attention to a clear discrimination between the right and left vagi. General conclusions have been drawn, with regard to vagus activity, which are applicable only to the left vagus, and in the earlier publications no attempt even was made to differentiate between vagus and accelerator nerves, so that functions peculiar to different nerves have been assigned to both vagi.

The idea that the vagus nerves may or may not exert contralateral effects was not put to the test of experiment till 1911, when Garrey (1) carefully considered the matter, using the whole and partially split heart of the turtle and recording his results graphically. His conclusions, which are very definite, are as follows. He asserts first, that the origin of the beat is to be found in the right caval veins, the inherent rhythmicity of which he showed to be greater than that of the sinus and auricles and then he shows that, in the whole heart, the right vagus affects chronotropically, through its action on the right veins, every part of the organ, while the left vagus may affect the whole, including the right veins, but that usually it affects the left veins, the sinus and the auricles, producing quiescence by decreasing the excitability, conductivity and contractility of the auricles, leaving the rhythm of the right veins unaffected. In the "ascidian preparation" of the turtle's heart, in which the beat travelled from right to left, very clear conclusions were arrived at, namely, that the right vagus stopped the whole preparation, while the left inhibited the left auricle and right auricle, the right basal veins still continuing to beat at their original rhythm. This, according to Garrey, is due to the fact that the left vagus does not affect the pace-maker, but acts upon structure similar to that upon which normal cardiac impulses act. The essential point determined is that the vagi act chronotropically on their respective sides, upon those parts which initiate the rhythm. This is the basis of the homolateral view of the action of the vagi, which has been so strongly brought forward by Garrey who, however, showed that such effects were not so clearly marked with regard to the auricles, and he states "that the experiments bring out clearly that in auricles as well as in veins and sinus the vagus effects may cross to the contralateral side."

Robinson and Draper (2), from an investigation of the action of the vagus nerves upon the human heart, corroborate previous findings on the mammalian heart, namely, that the predominant action of the right vagus is a control of rate, through its inhibitory action upon the normal pace-maker of the heart, and that of the left nerve is primarily a control of conduction from auricle to ventricle, through a direct inhibitory effect upon the conducting system and that in gauging the difference upon conductivity of the right and left vagi, the factor of heart rate must be taken into account. Again, with regard to auriculo-ventricular dissociations, it was shown that these are not caused by diminution in the conductivity, but are essentially due to the inherent high rate of rhythmicity of the ventricles, dissociation occurring as soon as vagus stimulation reduced the auricular rate below that at which the ventricles will contract by their own inherent rhythmicity. This viewpoint is supported by experiments of Rothberger and Winterberg (3) who demonstrated true nodal rhythm by stimulation of the right vagus and left accelerator nerves.

Cohn (4) in 1912 stated that negatively chronotropie effects may be obtained upon stimulation of the left vagus and he made this significant statement "that it is doubtful whether the distribution that has been described is as refined as is necessary to explain the results" he obtained.

With regard to the importance of heart rate in gauging dromotropic effects of the vagi, attention is drawn to a paper by Robinson (5) in 1916, in which he shows that there is no constant difference between the right and left vagus nerves in their action upon conductivity between the auricles and ventricles during auricular fibrillation.

The first report upon the electrical changes in the heart due to vagus stimulation was made by Gaskell (6). The type of experiment which he describes depends upon the peculiar arrangement of the vagus nerve in the tortoise heart, where a branch of the vagus runs with the coronary vein from the sinus to the base of the ventricle. This "coronary" vagus being free, the sinus and left auricle could therefore be cut off without damage to the nerve supply of the right auricle, thus allowing of a quiescent preparation. A demarcation current having been produced by "thermic section" and the right vagus stimulated, a deflection was obtained in the same direction as that of the injury current, indicating increased positivity of the uninjured part. These experiments were carried out by means of a d'Arsonval galvanometer.

Gotch (7) in 1887, using a capillary electrometer, could not obtain this vagus effect, the position of the meniscus, upon vagus stimulation, remaining at the position obtaining during diastole. It was suggested by Burdon Sanderson (8), during a discussion of this subject, that the capillary electrometer was probably not sufficiently sensitive to detect such slight changes of potential as had been detected by Gaskell by means of a much more sensitive instrument.

Einthoven (9) in 1908 criticised Gaskell's results and doubted their accuracy because, by means of the string galvanometer he was unable to detect any changes of potential whatsoever. That Gaskell was correct was clearly demonstrated in 1911 by Meek and Eyster (10), who with an Edelmann galvanometer, using the tortoise heart prepared according to Gaskell's method, obtained uniformly positive results. Theirs was an exceptionally clear corroboration of the Gaskell phenomenon, as evidenced by a rapid deflection of the string and its slow return to the original position. If the heart were beating, this prolonged slow fall of the string to its original level would be cut short by the first beat following upon inhibition and the return of the base line to the zero position would be rapid. That this happens in the beating heart was shown by Samojloff (11), who used the same type of instrument. The hearts of decapitated frogs were utilized, an injury current was produced by the application of a drop of 1 per cent KCl to the apex of the heart, and leads taken, one from the apex the other from an uninjured part of the heart. A monophasic variation was obtained with each beat, and upon stimulation of the right vagus the zero line was deflected in the direction of the injury current; the deflection was slow and emphasis was laid upon the fact that the return of the string did not begin until a contraction supervened.

PART I

Electrical changes associated with the action of the vagi

The fundamental principle involved in determining these changes depends upon the following acceptation.

With a demarcation current or injury current. In normal muscle excitation is evidenced by an increased negativity of the part involved and electrodes can be so arranged that such negativity gives rise to an upstroke of the string of the galvanometer. When in the heart an injury current is produced, the injured surface becomes electrically negative to the uninjured part and the difference of potential thereby occasioned gives rise to a deflection of the string in a direction opposite to that which denotes excitation in normal muscle. A wave of excitation passing over such a field would produce at the positive or uninjured part a decrease of its positivity with respect to the injured spot and therefore cause a rise in the direction of, but smaller than, that due to a normal contraction. If the vagus nerve be stimulated, its effect is to reduce the condition of negativity and therefore relatively increase the positivity, the result being a deflection of the string in the same direction as that of the injury current.

In the normal or uninjured condition. To obtain monophasic variations from the action of the heart muscle, one electrode must be placed on the heart, the other on a part of the body wall sufficiently removed from the heart to allow of the activity of all parts of the heart muscle being marked by an upward deflection. If now the electrical changes of the heart, due to its activity, are not totally inhibited, these will show themselves as upstrokes of reduced amplitude, arising from a zero line at a lowered level.

It may be assumed that whatever occasions the beat produces a sudden catabolic change, a change dependent upon a previous building up of excitability or, to use Gaskell's term, a process of assimilation. There is no reason to suggest that such a process of assimilation should be of such a sudden nature as that of the catabolic discharge or dissimilation; the deflection caused by the one may be wholly different from that caused by the other, the factor chiefly concerned being that of velocity. It is supposed that these anabolic changes are inaugurated by vagus action and therefore that electrical stimulation of the vagus increasing these, it should be possible, using a galvanometer of sufficient sensitivity, to detect the changes in potential arising therefrom.

During inhibition the heart suffers an alteration of its excitability and its activity is depressed. It may therefore be feasible to accept the interpretation of Samojloff's curves, that contractions may be resumed at an increased level of anabolism, provided the process has reached completion and stopped, as otherwise it is difficult to conceive of contractions supervening during a process which is essentially of the nature of an inhibition. If the building up of excitability or the inhibitory process has not reached its maximal development, then, to explain the breaking through of heart beats, one must assume that the vagi and the contractions act upon different mechanisms in the cardiac muscle. Gaskell (12), McWilliam (13), (14), and Roy and Adami (15) state that excitability is diminished during inhibition but all that they can prove is, that the heart in inhibition was inexcitable, and that, during a period when it was establishing a necessary condition such that the subsequent stimulus should produce a contraction, that is, a condition of excitability. Therefore their diminished excitability is associated with what may be regarded as a refractory period of inhibition. Just as there is a refractory period in catabolism so one postulates a similar condition in anabolism. This would support the idea suggested that the upstroke is indicative of the true inhibitory period, i.e., the time till maximal deflection is reached, after which the excitability of the tissue is such that stimuli may be effective, which stimuli, however, may be delayed over a longer or shorter period, according to the degree of diminution of conductivity, which is a manifest effect of both vagi.

The results of the experimental work to which reference has just been made, demonstrate clearly the occurrence of electrical changes in the heart during vagus stimulation. These changes then may be employed to determine the distribution of the vagi in the heart, to demonstrate the action of these nerves upon its various parts and to decide if possible by what means vagal impulses are propagated. To do this a preparation of the whole and also of the partially split heart has been used.

Experiments carried out with the d'Arsonval galvanometer

From previous work done, using the string galvanometer for determining the electrical changes occurring in the heart upon vagus stimulation, it was found that, in the normally beating heart, the string galvanometer is too sensitive an instrument to withstand the action current of the heart, when the string is slackened to that extent which is necessary to give definite evidence of the positive variations. With sensitivities such that 1 m.v. gives deflections from 5 to 10 cm., the results are in many cases not convincing. It was therefore determined, seeing that it was impossible to carry out these experiments upon a quiescent heart, to utilize a very sensitive d'Arsonval galvanometer and, by means of a rheotome, to place it in circuit with the heart only, during the very brief period of its quiescence between beats.

Methods

The rheotome. This consisted of a brass segmented wheel having one continuous central contact and two, one on either side of the former, in which there was placed a different length of fiber, so that by adjustment of these, any length of non-conducting material could be readily obtained. Thus by using three contacts, the two external being connected, a definite period of time could be obtained during which the galvanometer could be thrown into the circuit. This period was chosen so that the ventricular, auricular and sinus effects could be eliminated. The circumference of the rheotome was 500 mm., the gap was, after experiment, cut down to 45 mm.

The quiescent period of the heart. To throw the galvanometer into circuit at this point of the cardiac cycle, a simple make and break device was arranged whereby the relaxation of the ventricle completed the circuit between six storage cells and a solenoid, which lifted the catch checking the rheotome wheel. This allowed the wheel to rotate, the retaining pawl being so placed that immediately it was lifted upon ventricular relaxation, the galvanometer was put in circuit with the heart. The rotation of the segmented wheel was so arranged by a switch upon the power table, that one revolution was just completed within the period of the cardiac cycle. The pawl, which was dropped upon contraction breaking the circuit, stopped the wheel for a very brief moment, depending upon slight variations in the heart rate.

It was found that in the majority of cases it was necessary, in order to obtain a quiescent period, to cool the heart, because, in a heart beating at the rate of thirty per minute, the sinus was in action sometimes during a ventricular contraction, or following so closely upon ventricular contraction that the rheotome method was rendered of no avail. The sinus was cooled by means of a blind perfusion cannula, through which was maintained a continuous flow of ice-cooled water of a temperature of about 10°C. The cooled point was maintained upon a definite spot on the sinus, a spot previously determined as being the seat of highest rhythmicity.

The sensitivity and calibration of the galvanometer. The d'Arsonval galvanometer used in these experiments was the type "R" of the Leeds & Northrup Company, which had a sensitivity of 5×10^{-10} amperes per mm. and a voltage sensitivity of 0.5 mm. per microvolt, with a period of 5 seconds. Calibration of the instrument, with the rheotome running, gave readings for 0.1 m.v. of 7.3 cm. in 5 minutes and a return to 0.5 cm. from zero in 6.0 minutes. Therefore a deflection of 7.3 cm. = 0.1 m.v. and is equal, without the rheotome to a deflection of 22 cm. From this it is seen that to obtain full values with the rheotome method, it would be necessary for the vagus effect to last 5 minutes. From the rate of rise of the curves and from their magnitude it will be seen that the "vagus" effect is a very marked one, is maintained and is dissipated quickly by subsquent beats.

A difficulty which arises with the rheotome method is, after vagus stimulation, to cut out the prolonged beats of the auricle which usually encroach upon the quiescent period of the heart, even abolishing it, and so cause a sudden return of the beam of light, which may reach its original position or pass beyond it in two or three steps. One can, however, by operating the rheotome with a key, cut out the auricular beats occurring immediately after inhibition, the solenoid circuit being broken upon the first sign of movement in the basal veins.

Compensation of the injury current. With a very slowly moving coil galvanometer, with the time of stimulus very short, namely 0.18 second when the rheotome was making thirty revolutions to the minute, the response to small currents may be so small as scarcely to be noticed. In compensating, therefore, one must accurately gauge the period of the heart cycle in which no electrical effects from auricular beat are allowed to encroach upon the period of quiescence. The sinus effect is so small that deflections caused thereby can be ignored, but small inclusions of auricular effects would simulate over-compensation. This, as well as the opposite effect of under-compensation, must be guarded against, because the latter, if not accurately gauged, will give deflections in excess of those due to vagus stimulation, since a deflection denoting an increased positivity is an extension of that of the injury current.

Type of reading obtained. It is essential, if maximal deflections are to be obtained, to stimulate the vagus, maintaining the quiescence of the heart, till there is no further increase in the amplitude of the deflection. This may necessitate a vagus stimulation up to from 60 to 80 seconds, although maximal deflections are usually obtained well within this period. The type of reading resulting from this method is shown on the tracings, these records having been made by a device of Gesell (16), in which the beam of light can be followed and the curve recorded upon a moving drum. The record is in the form of a series of steps which are of varying sizes and are in the form of curves as shown in several tracings. It was not practical to follow accurately these fine swinging movements of the beam of light. The first few initial steps are steep; they then rapidly become less and less in size till, at the plateau of the curve, the small pendular movements synchronous with each revolution of the rheotome only are in evidence. The plateau may be maintained for a longer or shorter period and is, unless the rheotome is controlled by hand, suddenly terminated by the first auricular contraction.

Type of reading without the rheotome. In the partially split heart, where the wave of contraction sweeps from right to left, it is of course possible to prevent auricular or ventricular effects of the side under observation, from affecting the galvanometer, but it has been found that after 2 or 3 hours, when the activity of the heart has become considerably lessened, the ventricle has little effect upon the galvanometer, the sinus none at all and the auricular contractions show as small deflections, with a total amplitude of from 1 to 1.5 cm. With such a weakly contracting heart, beating at about sixteen per minute, the mean of these deflections can be taken, because the beats are usually so weak that any cumulative effect takes a considerable time to show any alteration in the mean level of the beam of light. Thus the changes occurring upon vagus stimulation are quite easily discernible.

The whole heart

Table 1 shows that the right vagus is always markedly active upon the right auricle and in many cases very slightly less so upon the left auricle. The point of note with regard to the left auricle is that, in about a fourth of the cases, the right vagus is more effective than the left, and generally the left vagus is always less marked in its action upon the left auricle than is the right vagus upon the right auricle, while upon the right auricle the left vagus is, with three exceptions, decidedly weaker in action than either the right or the left vagus acting upon its respective side. The tracings reproduced here as figures 1 to 5, with attached explanatory notes, illustrate the basis upon which these conclusions rest. All were obtained through the d'Arsonval galvanometer.

TABLE 1

Results with the d'Arsonval galvanometer, with an injury current

	RIGHT VAGUS		LEFT VAGUS		REMARKS
	Right auricle	Left auricle	Left auricle	Right auricle	
	cm.	*cm.*	*cm.*	*cm.*	
1	4.5	3.5	5.5	3.0	Heart cooled. Rheotome rate 24 revolutions per minute
2	7.5	5.0	4.0	3.5	Heart quiet with muscarine; no rheotome
3	4.5	4.0			
4		10.5	5.5		Heart rate 16 per minute
		11.5	5.0		Temperature 10°C.
5		9.0	8.0		Without rheotome; heart quiescent; right auricle cut away
		8.5	9.5		
		8.5	11 0		
		9.0	9.5		
6	2.5			1.0	Gaskell's preparation; coronary vagus intact
	1.5			0.5	
7		0.8	1.0		Sinus cut off from right auricle; coronary vagus intact; left auricle quiet for short periods
		1.0	1.5		
8		7.8	8.8		Heart rate 15 per minute, temperature 10°C.
9		8.5	6.0		Heart rate 20 per minute; heart cooled
		6.5	5.5		
10	6.5	6.5	4.5	3.5	Heart rate 20 per minute; heart cooled
11	6.5	5.0	4.0	4.5	Heart rate 20 per minute; heart cooled
12	6.2	5.5	4.5	5.0	Heart rate 20 per minute; heart cooled
13	3.5	2.5	3.3	2.8	Heart rate 20 per minute; heart cooled
14	5.5	3.0	2.5	2.0	Heart rate 20 per minute; heart cooled
15	2.0		2.0		Heart completely split
16	3.5		2.0		Heart completely split
	2.0		1.0		
17	4.8		3.3		Heart completely split
	4.0		2.5		
18	5.5	5.5	4.2	4.0	Heart cooled
19	2.2	1.8	1.9	1.6	Heart cooled
20	2.5			2.0	Heart cooled
21	3.3	2.0	3.2	2.0	Heart cooled
22	2.1	1.0	2.2	0.6	Heart cooled
23	1.8	1.2	1.4	0.9	Heart cooled
24	1.6			0.8	Heart cooled
25	2.9	2.0	1.9	1.2	Without the rheotome
26	2.6	2.3	2.1	2.0	Without the rheotome

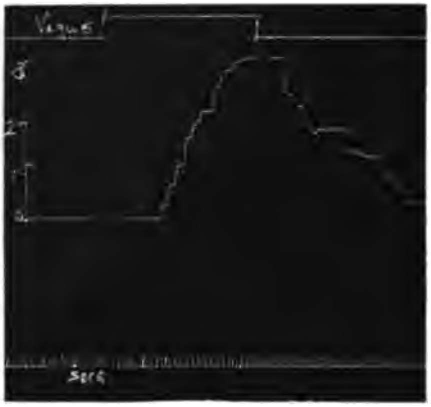


Fig. 1. Whole heart; right auricle, injury current; right vagus stimulation. This is a typical result of the rise and fall during the quiescent period of the heart. Here the only part of the curve not due to vagus activity is the first downstroke occasioned by the commencing auricular beat. The vagus was stimulated for 36 seconds, the deflection obtained being 3.2 cm.

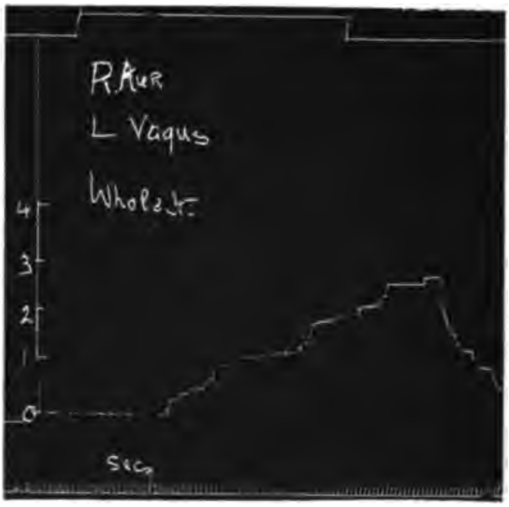


Fig. 2. Whole heart; right auricle, injury current; left vagus stimulation. This record shows the steady rise step by step indicating increased positivity of the left auricle. The inhibition of the left side was maintained for a period of 58 seconds, while stimulation of the vagus lasted for 60 seconds, producing a maximal deflection of 2.6 cm.

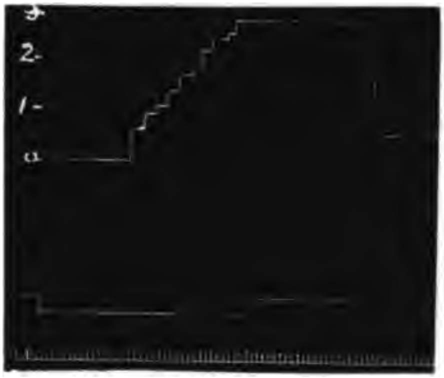


Fig. 3. Whole heart; left auricle, injury current; left vagus stimulation. This is a very good example of the steady step-like movement of the beam of light. The vagus was here stimulated for 36 seconds, the duration of the rise was 24 seconds and the maximal deflection was 2.7 cm.

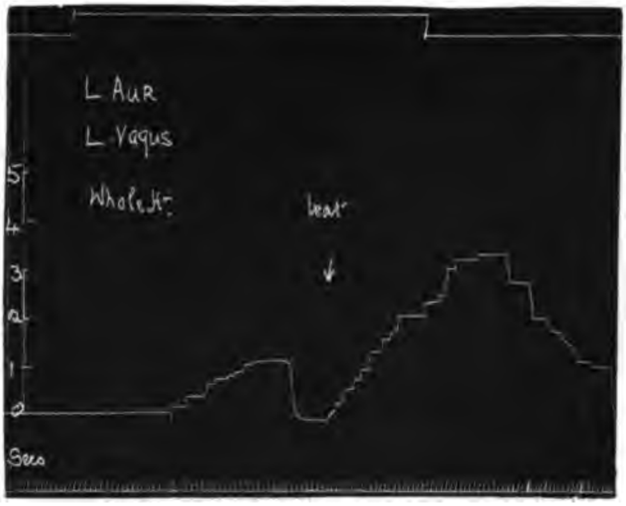


Fig. 4. Whole heart; left auricle, injury current; left vagus stimulation. This tracing is of interest in showing the effect of one beat of the heart breaking through in a short inhibition, which is then followed by a steeper and longer rise giving a total deflection of 3.2 cm. Stimulation of the vagus was continued for 64 seconds and there was no apparent sign of shifting of the electrodes or temporary stoppage of stimulation to account for the single beat.

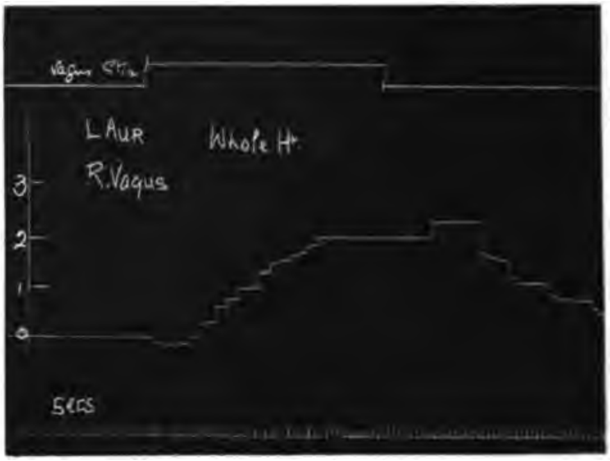


Fig. 5. Whole heart; left auricle, injury current; right vagus stimulation. The total deflection of 2.0 cm. was obtained in 30 seconds. The rise and fall are both typical, the fall below the original base line being due, probably, to a diminution of the demarcation current.

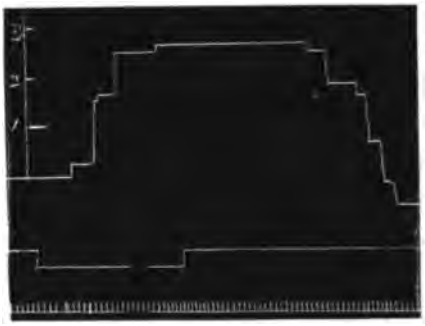


Fig. 6

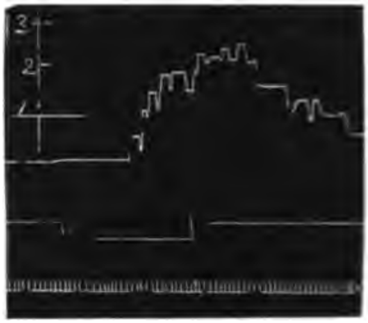


Fig. 7

Fig. 6. Partially split heart; rheotome; right auricle, injury current; right vagus stimulation. In this case the injury current was not compensated. The vagus acted for 12 seconds and gave a maximal deflection of 2.6 cm.

Fig. 7. Partially split heart; rheotome; right auricle, injury current; right vagus stimulation. Here the pendular movements were followed as accurately as possible. This record shows exactly the type of movement which is performed by the beam of light upon the scale, as the galvanometer responds to every brief impulse which it receives with each revolution of the rheotome. The inhibition was maintained for 26 seconds and the rise was completed in 15 seconds, the total deflection being 2.3 cm.

The partially split heart

With the rheotome. The type of results obtained from the partially split heart when the d'Arsonval galvanometer is put into circuit by means of the rheotome is seen in figures 6 and 7 and table 2, and described in the legends to the figures.

Types of deflections obtained without the rheotome. Using the rheotome, the results have, in the case of the action of the vagi on the contralateral sides of the heart, been invariably negative. In fact there is to be seen a slow movement of the beam of light in the direction opposite to that indicating a positive variation, due either to a diminution of the injury current or to the effects of weak contractions from parts of the heart, other than the auricle under obsevation. The best and most decisive results in the partially split heart have been obtained, without the rheotome, 2 to 4 hours after opening the pericardium and about ½ hour after making the sagittal section, because by this time the beats, running from right to left, have lost much of their vigor and rapidity.

TABLE 2

Results with the d'Arsonval galvanometer, with an injury current; split heart

	RIGHT VAGUS		LEFT VAGUS		REMARKS
	Right auricle	Left auricle	Left auricle	Right auricle	
	cm.	*cm.*	*cm.*	*cm.*	
1		3.0 2.0	1.5		These readings correspond with those obtained previous to sagittal section. Therefore (?) faulty section
2	4.5	0.0	6.5	0.0	
3	4.0	0.0	5.0	0.0	
4		0.5	3.5	0.0	
5		1.0	4.0	0.0	
6		0.0	5.0	0.0	
7	3.5	0.0	2.0	0.0	
8	3.0	0.0	2.5	0.0	
9	5.0	0.5	3.5	0.0	
10	2.0	0.0	2.0	0.0	
11	2.8	0.0	4.4	?	
12	3.3	0.0	3.2	0.0	

The current of injury is not compensated and from the tracings (figs. 8 to 13) it will be seen, that the movements, due to contractions, are very small indeed.

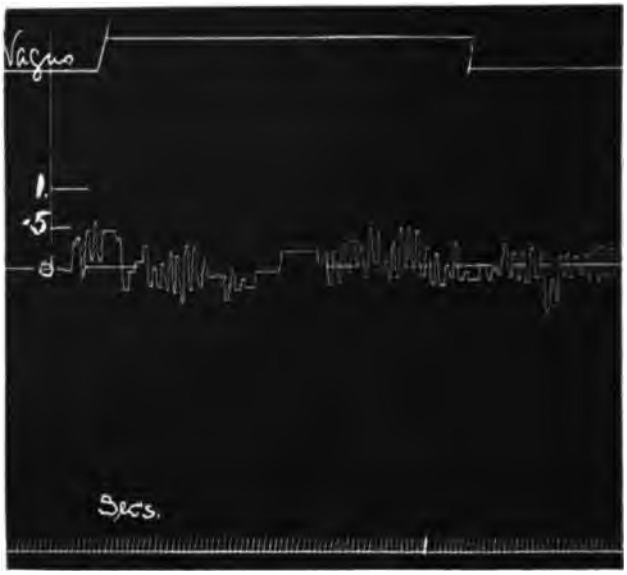


Fig. 8. Partially split heart; without rheotome; right auricle, injury current; left vagus stimulation. The movements of the beam of light were successfully followed in this case and it will be noted that there is neither inhibition of the right auricular beat nor alteration of the base line due to a stimulation of the left vagus lasting 52 seconds.

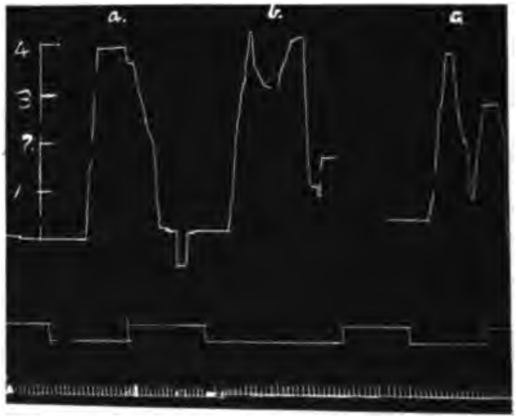


Fig. 9. *a*, *b*, *c*. Partially split heart; without rheotome; right auricle, injury current; right vagus stimulation. These three are typical of results obtained without the use of the rheotome. The height and rapidity of the deflections are comparable to those obtained by Gaskell, and show a definite electro-positive change. The deflections have a maximum of 3.8, 4.0 and 3.3 cm. with a time of 3.0, 4.0 and 3.5 seconds, respectively. The tendency, even in a weakly beating heart, for the "vagus" effect to be repeated, is seen in these tracings.

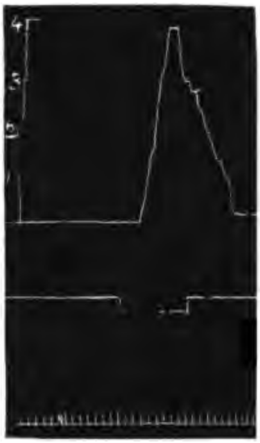


Fig. 10. Partially split heart; without rheotome; left auricle, injury current; left vagus stimulation. The action of the left vagus upon its own side, as graphically shown here, is similar to that obtaining in the whole heart. The response is rapid and the time taken to attain a maximal deflection of 3.8 cm. is 4.0 seconds.

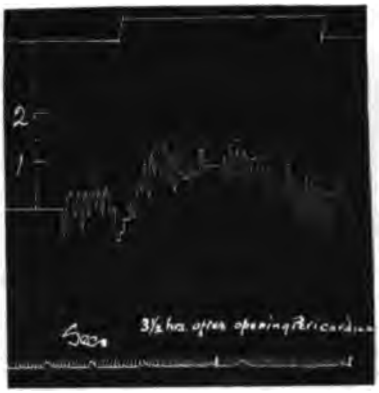


Fig. 11. Partially split heart; without rheotome; left auricle, injury current; left vagus stimulation. This shows activity of the left vagus upon the left auricle three and one-half hours from the commencement of the experiment. The pendular movements are due to the right auricular beats.

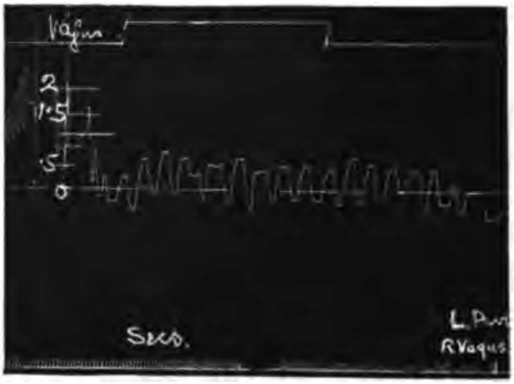


Fig. 12. Partially split heart; without rheotome; left auricle, injury current; right vagus stimulation. The survival of the left auricular contractions in the partially split heart, upon right vagus stimulation, is clearly seen here. The more rapid beats due to the right auricular rhythm are seen at the beginning of the tracing and their almost immediate inhibition upon right vagus stimulation is clearly shown, the slower left auricular rhythm remaining. That in this "ascidian" preparation the right vagus has no effect upon the uniform position of the base line, which is the mean of the auricular deflections, is demonstrated in this experiment, where the right vagus stimulation lasted for 58 seconds.

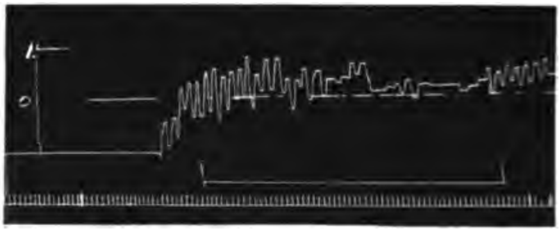


Fig. 13. Partially split heart; without rheotome; left auricle, injury current; right vagus stimulation. The fact that the right vagus has no action on the left auricle in the partially split heart is again shown here. After the deflection of the injury current was obtained, the movements of the beam of light were followed and the right vagus stimulated for 60 seconds. No alterations in the base line took place. The right auricular contractions were inhibited and the latter half of the tracing shows only the left auricular deflections, which with their slower rhythm come into evidence toward the end of the tracing. This occurs after a period of stand-still of very brief duration, during which time the inherent rhythmicity of the left auricle becomes established.

PART II

The quantitative effects of the vagi

The foregoing results with their demonstration of crossed vagal effects suggested a quantitative study of these.

To determine what quantitative vagus changes may occur on both sides of the heart the graphic method was used and the curves plotted on coördinate paper. As the rheotome revolved at a rate of either twenty or thirty revolutions per minute, and as both vagi, in most of the experiments, completely stopped the heart, the repeated contacts were made at regular intervals, thereby giving one a means of comparing the rate of deflection of the beam of light step by step, each step denoting a definite period of time during which the galvanometer was in circuit with the preparation. To arrive at some idea as to the intensity of the vagus action, it is necessary to compare the deflections, using as a standard a definite period of time during which the nerves are stimulated, and to do this the most convenient method is to take a number of steps or a number of revolutions of the rheotome, but not more than that required by the lowest curve to attain its maximal deflection. Without the rheotome, to take a definite height as a standard and compare the time required to reach it, would be admissible, but with the rheotome a standard height precludes any comparative conclusions, because it manifestly gives the slower effect the advantage of a greater number of contact periods.

The curves are plotted for the position of the beam of light either every 5 seconds or for every revolution of the rheotome; the abscissae denote time in seconds and the ordinates deflections in centimeters. The stimulation of the vagus, marked by a signal, was usually continued till the maximal effects were obtained, the time was recorded by a Jacquet chronograph and the records were made by Gesell's method already referred to. The results obtained can best be presented as descriptions of the curves.

Figure 14. Here the right vagus was stimulated for 36 seconds, and the electrical change commenced 13 seconds after stimulation began, and in 38 seconds the maximal deflection had been obtained. The curve both in its gradient and height is typical of the right vagus effect. In 15 seconds the beam of light had risen 2.9 cm., after which the change became more and more gradual, till 10 seconds later, the maximal deflection of 3.3 cm. was attained, where it was steady for 5 seconds and then fell at first quickly, later more and more slowly

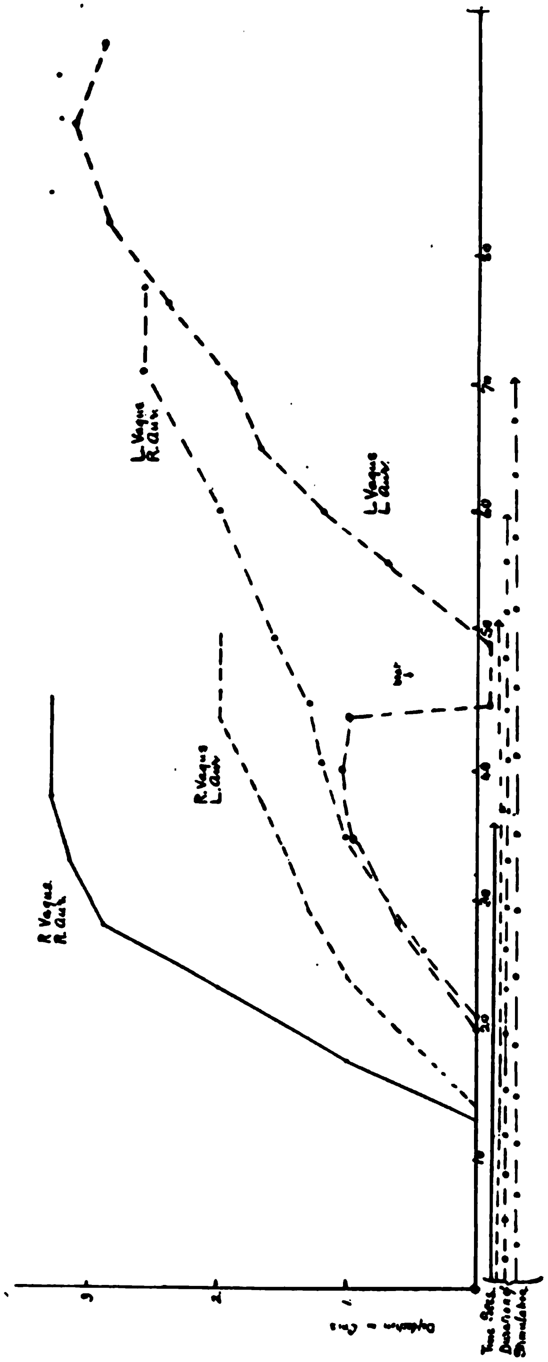

R Vagus
R. Aux
R. Vagus
L. Aux
L Vagus
R. Aux
L Vagus
L. Aux
Deflection – cms
1
2
3
10
20
30
40
50
60
70
80
Time Secs
Duration of Stimulation

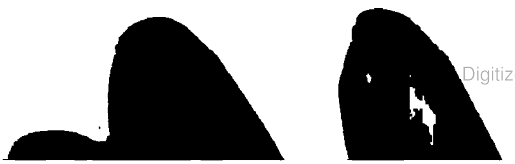
Fig. 14

as the base line was approached. The right vagus effect upon the left auricle shows a change commencing as soon almost as that of the right vagus upon the right auricle. The gradient of the electrical change is less marked though quite as definite as that of the homolateral effect. The total stimulation lasted 52 seconds and the maximal height of 2 cm. was reached in 30 seconds. The left vagus in this experiment had a longer latent period but, like the right vagus, the period was practically the same for both sides, 20 and 21 seconds respectively. This is the case previously mentioned, in which a beat was interpolated 24 seconds after inhibition had been produced. The action current of the heart caused an immediate return of the beam of light to just below zero, inhibition was again produced and in 5 seconds it is evident and much more markedly so than in the previous rise. Comparing the left vagus effect upon the right and left auricles, it is seen from this figure that the second rise due to the left vagus is steeper than that of the left vagus effect upon the right auricle. This is the usual result obtained, though not without exception. The maximal rise and the time taken for each vagus on both sides of the heart are as follows:

	cm.
Right vagus acting upon right auricle for 18 seconds...............	3.3
Right vagus acting upon left auricle for 30 seconds................	2.0
Left vagus acting upon left auricle for 40 seconds..................	3.2
Left vagus acting upon right auricle for 49 seconds................	2.0

Figure 15. For comparative purposes the deflections occurring upon each of five consecutive revolutions of the rheotome are plotted. The marked ascendancy of the right vagus upon the right side of the heart is well shown; also from the graph of the second rise of the left vagus upon the left side, which has been traced in for comparison in the position of the original rise, it can be seen that the very definite effect upon its own side is greater than the right vagus effect upon the left both in maximal deflection and in rapidity of its rise. The left vagus action upon the right side is the least marked of all and this record, with the second curve of the electro-positive change of the left vagus transcribed, sums up very clearly the various effects occasioned by both vagi, each acting upon both sides of the heart.

Noting the deflections after five revolutions of the rheotome, the heart being inhibited by both vagi and the time interval between the stimuli being the same for all, namely, 2 seconds, the galvanometer being in circuit with the heart for a period of 0.18 second, we have the following results:



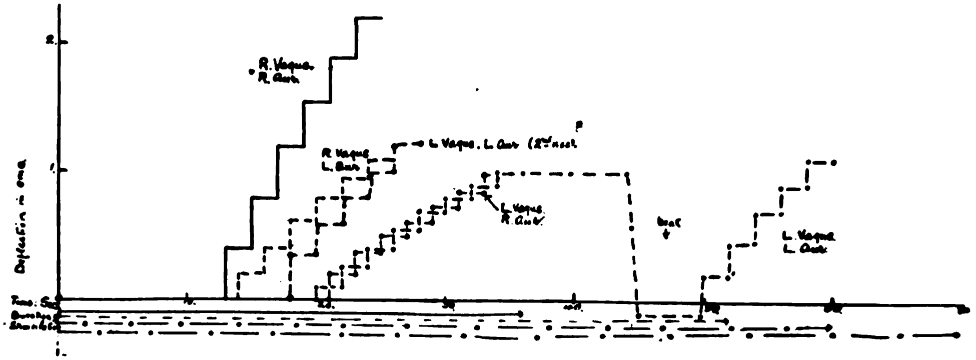


Fig. 15

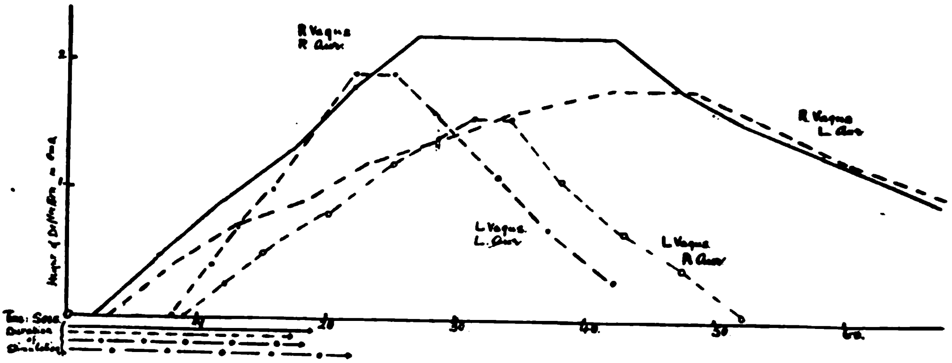


Fig. 16

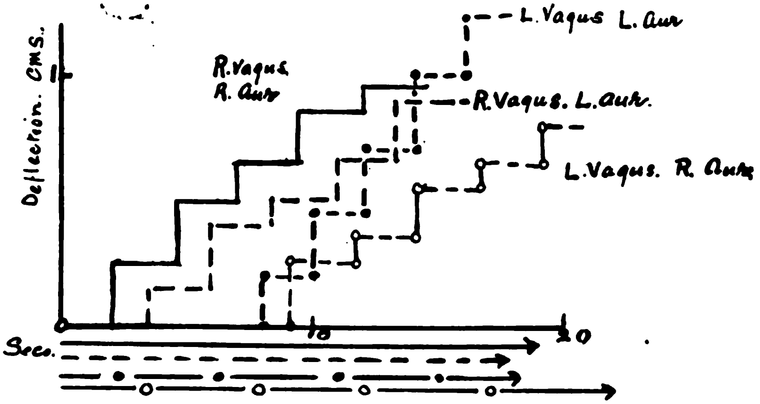


Fig. 17

	cm.
Right vagus acting upon right auricle	1.9
Right vagus acting upon left auricle	0.9
Left vagus acting upon left auricle	1.1
Left vagus acting upon right auricle	0.7

Figure 16. As in the preceding record, the greater latent period of the left vagus as compared with that of the right is shown and here the left vagus action upon its own side is very definite, especially with regard to the velocity of the change, in which in this case it exceeds that of the right vagus upon the right side. The slower contralateral effects, though of scarcely less magnitude are noteworthy, when one recalls what has been suggested with reference to the anatomical distribution of the vagi. The maximal deflections and the time taken for their completion are as follows:

	cm.
Right vagus acting upon right auricle for 25 seconds	2.2
Right vagus acting upon left auricle for 39 seconds	1.8
Left vagus acting upon left auricle for 14 seconds	1.9
Left vagus acting upon right auricle for 22 seconds	1.5

Figure 17. Comparing these effects in four steps of 2½ seconds each, with the exception of the left vagus upon the left auricle in which each step equals 2 seconds, one sees from the record that they are very similar to those in the preceding case. The right vagus was very active, having, for both sides of the heart, a latent period of 2 and 3 seconds respectively. The difference in the velocity of the change effected is not so marked in the first 10 seconds as subsequently; yet the difference in the gradient of the activities of the left vagus upon the left and the right auricle is well marked. The latent period of the left vagus, while greater than that of the right, is, comparable to the right vagus, practically the same for both sides of the heart, namely 8 and 9 seconds for the left and right sides respectively. In this experiment the stimulation of the nerves lasted approximately 20 seconds in each case. The deflections from five revolutions of the rheotome were as follows:

	cm.
Right vagus acting upon right auricle	0.9
Right vagus acting upon left auricle	0.7
Left vagus acting upon left auricle	1.0
Left vagus acting upon right auricle	0.6

Figure 18. This is a record of the only instance in this series in which stimulation of the left vagus nerve did not stop the heart beat but caused a progressive slowing of the rate from 30 to 15 beats per minute

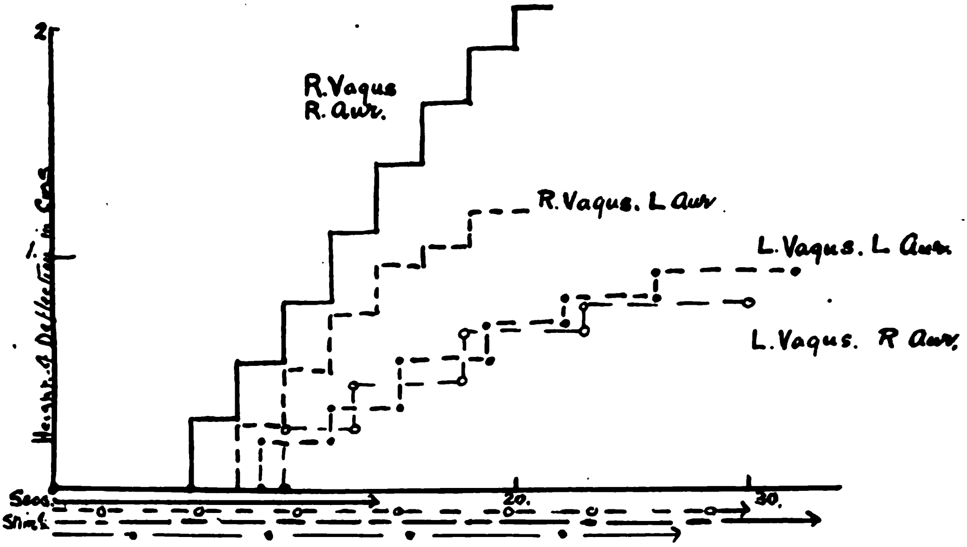

2
1.
Height of Deflection in Cms
R.Vagus
R. Aur.
R.Vagus. L Aur
L.Vagus. L Aur
L.Vagus. R Aur.
Secs.
Stim.
20.
30.

Fig. 18

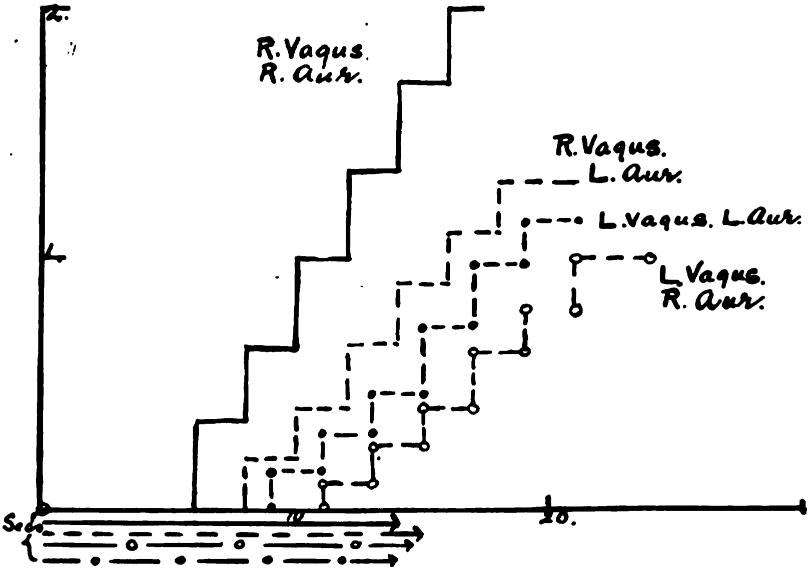

2.
1
R.Vagus.
R. Aur.
R.Vagus.
L. Aur.
L.Vagus. L.Aur.
L.Vagus.
R. Aur.
Secs.
10
20.

Fig. 19

in a period of five beats. After the sixth beat there was a pause of 7 seconds, the heart then became tumultuous, big, forcible contractions of the auricles and ventricle, of long duration, following in no regular sequence. After a series of three or four of these had passed, it was again possible to place the galvanometer in circuit with the preparation during the quiescent period, the duration of which became longer and longer till it was again encroached upon by a similar series of irregularly timed, forcible, heaving contractions. While a similar alteration in the rhythm was produced when leading from the right auricle and stimulating the left vagus, the maximal effect was obtained in four steps. The nerve was stimulated for 31 seconds and the maximum reached in 12 seconds. The results for all four activities were as follows:

	cm.
Right vagus acting upon right auricle for 14 seconds	2.1
Right vagus acting upon left auricle for 10 seconds	1.2
Left vagus acting upon left auricle for 32 seconds	1.6
Left vagus acting upon right auricle for 12 seconds	0.8

Figure 19. These curves denote the positive variation obtained with six revolutions of the rheotome, this number being that taken by the smallest curve, left vagus acting upon right auricle, to reach its maximal height.

	cm.
Right vagus acting upon right auricle	2.0
Right vagus acting upon left auricle	1.3
Left vagus acting upon left auricle	1.2
Left vagus acting upon right auricle	1.0

Figure 20. In this case the maximal deflections were recorded step by step with the rheotome revolving once every 2 seconds. The vagi produced complete stoppage of the heart in the times noted on the chart and the deflections were remarkably regular in their rise and bear out what previous records have shown, namely, that the right vagus is the most predominant in action upon the right side of the heart, the left vagus less so on the left side, while here the usually parallel effects of the right vagus upon the left auricle and the left vagus upon its own side, are clearly demonstrated. The deflections for fifteen steps, that is for 30 seconds, were as follows:

	cm.
Right vagus acting upon right auricle	4.5
Right vagus acting upon left auricle	3.6
Left vagus acting upon left auricle	3.6
Right vagus acting upon right auricle	2.7

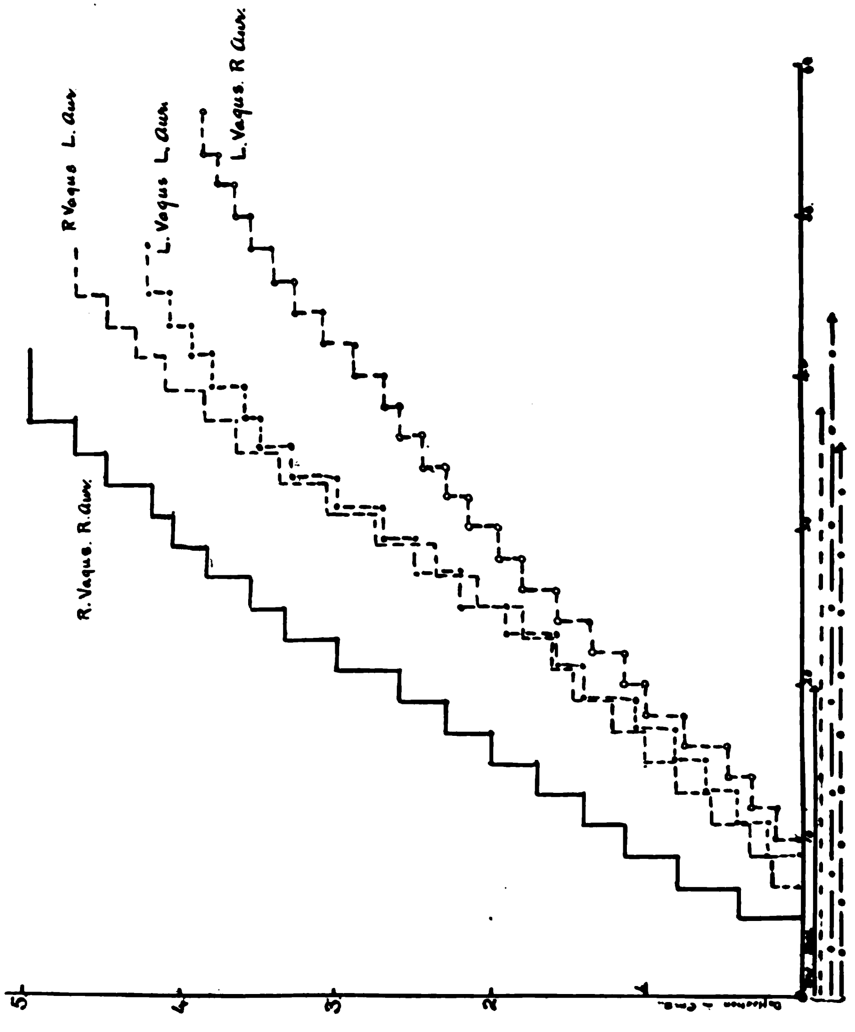


Fig. 20

Results without the rheotome. Figures 21 and 22. These records are given as typical of the curves obtained with a practically quiescent heart, in which deflections caused by electrical changes of weak contractions could be disregarded. These results are from hearts which have been bloodless for from 3 to 4 hours. The curves are plotted for the position of the beam of light every second and the duration of the stimulus is practically 10 seconds in all cases. One again sees the dominant effect of the right vagus, with a latent period of 5 seconds and 7 seconds for the respective sides of the heart in both cases. The maximal height is 2.6 cm. and 2.9 cm. respectively. The right vagus effects upon the left auricles are remarkably alike in both cases, the time taken to reach the maximum being 6 and 5 seconds, and the maximal height attained being 2.3 and 2.0 cm., respectively. Such an effect, compared with that obtained from the left vagus acting upon the left auricle, shows the resemblance between the crossed effects of the right vagus and the homolateral effects of the left vagus, with regard to the elec-

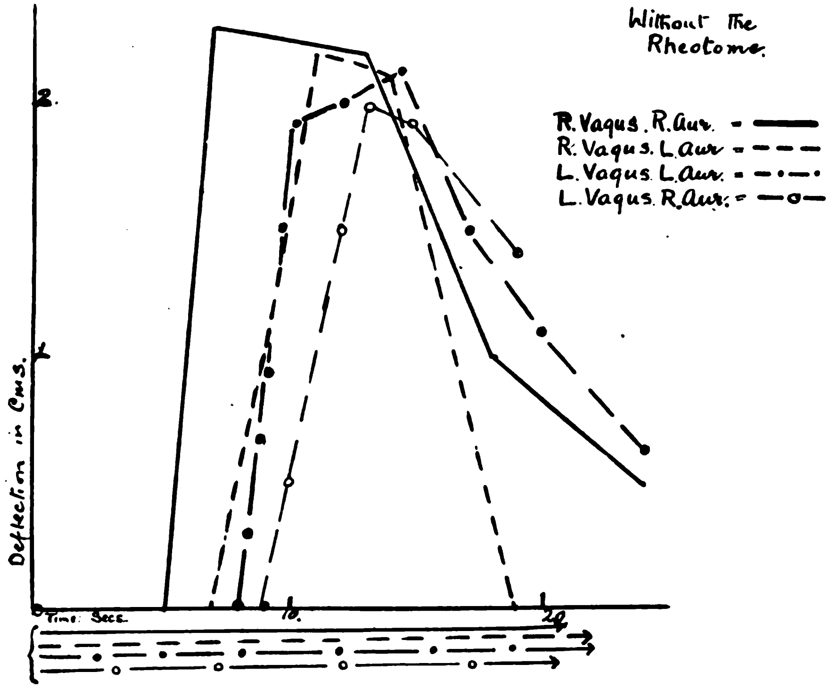


Fig. 21

trical changes they produce in the left auricle. The rapidity with which the change is produced in the left auricle is greater for the left than for the right vagus. From the latent periods and the heights of the curves, one sees that the crossed effects are slightly less rapid in their inception and in the velocity of their action. It is possible that the latent period, in obtaining crossed effects, is directly proportional to the degree of the contralateral distribution of the vagi. From the usually short lapse of time, 1 to 3 seconds, occurring in the production of the crossed effects, either by the right or the left vagus nerves, one must conclude that, anatomically, the crossed distribution of each vagus may, in many instances, be a liberal one.

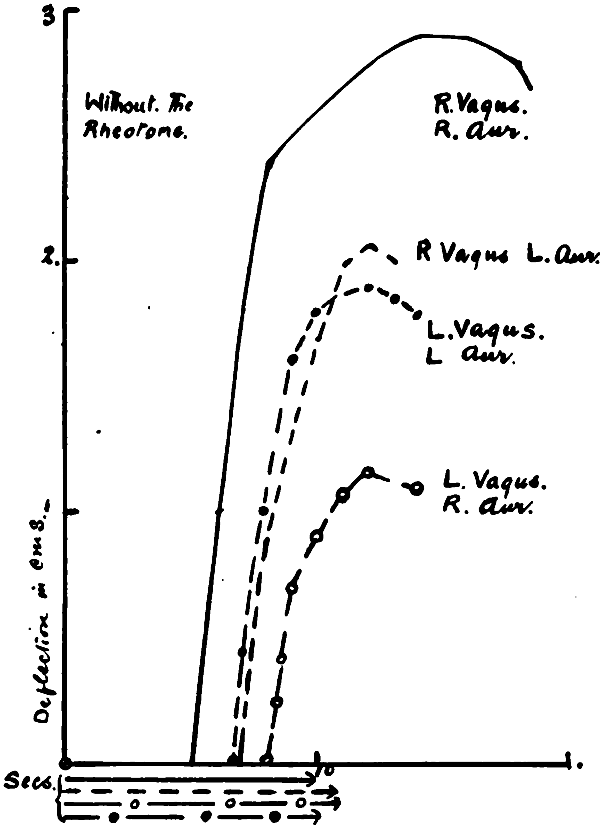


Fig. 22

With regard to the quantitative effects of the vagi upon the whole heart, the results may be summed up in a table of the various effects reduced to a percentage of the sum total:

Right vagus acting upon right auricle	40.9	26.6	35.0	36.3	31.2	30.0
Right vagus acting upon left auricle	20.4	21.9	21.4	23.6	25.0	24.3
Left vagus acting upon left auricle	23.6	30.1	29.5	21.8	25.0	25.7
Left vagus acting upon right auricle	15.0	20.3	14.2	18.1	18.7	20.3

This gives an average result of:

Right vagus acting upon right auricle	30 to 40
Right vagus acting upon left auricle	20 to 25
Left vagus acting upon left auricle	25 to 30
Left vagus acting upon right auricle	15 to 20

DISCUSSION

The experiments carried out with the d'Arsonval galvanometer described in the first part of this paper, corroborate the work of previous investigators with regard to the presence, in the heart muscle, of electrical changes, which are brought about by stimulation of the vagus nerves. It has been shown that these changes can be produced by each vagus nerve acting upon the opposite side of the heart. From the figures quoted in table 1 and from records shown of experiments upon the whole heart, it is evident that the action of the vagi is not mainly homolateral. As far as the vagus effect is distinguished by an increased positivity, by a change in potential, one must conclude that each vagus nerve exerts its greatest effect upon its own side, and upon the opposite side an effect which, generally, is definitely less than, but may, in the case of the right vagus, be almost as great as that of its homolateral action. In comparing the two vagi, it is seen from these results that the right vagus produces a much greater contralateral effect than does the left; that the effect of the right vagus upon the left auricle is generally less than that of the left vagus upon its own side. In only two cases did the left vagus acting upon the right auricle produce an effect greater than that of the homolateral action of the right vagus. It is seen that the contralateral effect of the left vagus is undoubtedly the weakest or least marked of all four activities studied.

These results do not substantiate Garrey's conclusions with regard to the preponderantly homolateral effects of the vagi. There may be a tendency to over-emphasize the homolateral view. In Garrey's case this may have resulted from his assumption that the beat originated in the right caval vein and from the fact that he could find no chronotropic effects thereon with left vagus stimulation. He states "that the left vagus is less effective upon the normal rhythm of the heart than the right vagus, due to the fact that it does not innervate the right basal vein;" and again he states "that crossed effects can be obtained, in some cases even to chronotropic effects, on the right veins by action of the left vagus."

If the left vagus can affect the center of rhythmicity in the left side of the partially split heart, is it not, from these results and from Garrey's suggestion, justifiable to conclude that the left vagus can or may, as a usual function, affect the pace-maker or center or centers of rhythmicity in the right auricle in the whole heart?

That such may be the case, more or less marked according to the degree of crossed distribution, is strongly suggested by the results recorded above, in which, in almost every case, left vagus stimulation quickly arrested the beat of the heart. As there were no evidences of block one must conclude that this is due to an effect upon the center of rhythmicity and that to stop the heart the vagus must act upon all heart tissue which is inherently rhythmical. Thus only, in those cases of complete stoppage, can left vagus inhibition be explained and this point of view demands a contralateral distribution of both vagi. With regard to the partially split heart, it is shown from the figures in table 2 and from the tracings briefly referred to, that here only are the effects of the vagi strictly confined to their respective sides.

This raises the question of the conduction of crossed effects which, according to Garrey, occurs by means of nerve. In the turtle, the crossed effects obtained in the whole heart must pass to the auricle by way of nerve fibers located in the tissue of the sinus. If the vagus effects were the result of a general conduction by means of both muscle and nerve, then, seeing that the muscle wave spreads around the sagittally split heart from right to left, there should be no reason why the vagus effect should not so pass.

In the partially split heart right vagus stimulation stops the whole initially, then the left sinus assumes a controlling rhythmicity for the left side, and if the right side be maintained in inhibition for some time, the beats originating in the left sinus will travel from left to right, but they will not pass from the ventricle to the right auricle. The passage of such contraction impulses shows that no vagus control is exercised upon the ventricle in the turtle. Also, it must be concluded that in the partially split heart no vagus effects can be transmitted across from the right to the left side of the heart, since there is no alteration in the electrical potential of the injury current of the left auricle when the right vagus is stimulated.

In concluding that the ventricular muscle of the turtle plays no part in transmitting vagus effects, it must be borne in mind that the reason may not necessarily lie in the ventricular tissue but in the inability of junctional tissue to transmit these effects. That vagus effects do not spread by way of the ventricle would not preclude such spread from right auricle to left auricle through cardiac tissue proper. Erlanger (17) and Erlanger and Hirschfelder (18) have shown that, after clamping the bundle of His in the dog and thereby producing partial or complete heart block, it is possible to demonstrate some action, though slight, of the vagus upon the ventricle. This goes to show that vagal impulses to some extent travel by routes other than those which subserve normal physiological conduction of the cardiac impulses.

That these impulses may not be associated with ordinary nerve fibers is suggested by the work of Meek and Leaper (19), who show that conduction in the heart, either by nerve fiber or by skeletal muscle, manifests no marked difference in the degree of compression necessary to destroy it. Garrey, in a study of the dissociation of inhibitory nerve impulses, states, "that if normal physiological conduction from sinus to auricle proceeds along nervous paths the blocking of these paths should at the same time block other nervous paths, including all vagus fibers which pass to the auricle through the clamped area, and through this area only." He shows that this is not the case; that compression sufficient to establish complete sino-auricular block, may not interfere with the passage of vagus impulses. This would indicate that if vagus effects are to be transmitted they must pass by way of tissue peculiar to them. That such effects do not spread through muscle tissue is proved by the larger effect upon the corresponding side in the whole heart. This rules out the idea that junctional tissue may be the cause of a non-transmittal of these effects in the partially split heart.

From these results one is led to the conclusion that there is no case for the spread of vagus effects by non-specific tissue, either muscular or nervous, but only by tissue hypothetically peculiar to vagus conduction. It would seem, from the results both in the normal and partially split heart, that for crossed effects in the whole heart, it is not a case of strong or weak stimuli affecting muscle fibers or nerve network, but rather a case of stimuli efficient enough to pass along paths of vagus conduction, the degree to which contralateral function is served depending upon the richness of the anatomical distribution.

With regard to the distribution of both vagi, evidence for a rich contralateral supply to the heart is borne out by this work and in support of this view reference has been made to the work of Cohn and of Robinson. The latter investigator showed that in gauging the action of the vagi upon cardiac conductivity, the rate of the heart had to be taken into account. From this it is seen that, in judging both of chronotropic and dromotropic effects of the vagi, the factor of greatest importance is the action of both right and left vagus nerves upon the center or centers of rhythmicity.

If it is assumed that the vagus acts by reducing the general reactivity of the tissue it happens to innervate directly, then the observation frequently recorded in the literature that left vagus stimulation often blocks the transmission of the contraction wave may be accounted for upon the basis of differences in the distribution of the two vagi with respect to the pacemaker. If in a given case the left vagus innervates the whole of the base of the heart excepting the pace-maker while the right vagus acts upon both parts equally, then stimulation of the left vagus will reduce reactivity and consequently the conductivity of the auricles, but not the rate of impulse initiation. The result would simulate the diminution in conductivity. Stimulation of the right vagus would produce the same reduction in reactivity and of the same parts but at the same time would slow impulse initiation. As a result the interval between successive impulse conductions might become long enough to permit the subjacent tissues, despite their lowered reactivity, to carry every impulse that came from the pace-maker. If the beats could be maintained at their original rate while the right vagus was being stimulated, a diminution of conductivity similar to that produced by left vagus stimulation would become manifest.

CONCLUSIONS

It is concluded that the positive variation of the demarcation current that develops during vagus stimulation is a phenomenon, not due to, although usually associated with, stoppage of the heart. This is shown by the facts:

1. That in the quiescent heart the electro-positive change is obtained.
2. That no electro-positive change is obtained, when in the partially split heart the left auricle temporarily stops beating upon right vagus stimulation.

Thus, looking at the question from two totally different standpoints, we have clear evidence that the electrical change indicative of inhibition is not occasioned merely by the cessation of muscle activity.

It is also concluded that in the turtle the distribution of the vagi through the base of the heart is bilateral, but is not uniform in all parts. In general the relative intensity of action is as follows: Right vagus on right auricle > right vagus on left auricle = left vagus on left auricle > left vagus on right auricle.

The greater tendency, noted in the literature, of stimulation of the left vagus to produce block is not necessarily due to a selective action of this nerve upon the conducting system; the result can be explained quite as well through the relatively slight action of the left nerve upon the pace-maker, which is located on the right side of the heart, while the reactivity of the remainder of the heart is reduced.

I am greatly indebted to Doctor Erlanger for his continued interest in this problem, and for much helpful and very suggestive criticism.

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THE INFLUENCE OF GLANDS WITH INTERNAL SECRETIONS ON THE RESPIRATORY EXCHANGE

I. Effect of the Subcutaneous Injection of Adrenalin on Normal and Thyroidectomized Rabbits

DAVID MARINE and C. H. LENHART

From the Department of Experimental Medicine and the Department of Surgery, Western Reserve University, Cleveland

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The demonstration by Asher and Flack (1), (2), (3) that stimulation of the laryngeal nerves in rabbits with intact thyroids increases and prolongs the rise in blood pressure following the intravenous injection of a given amount of adrenalin has provided the first concrete evidence of a thyroid-adrenal relationship. The Goetsch (4) test in exophthalmic goiter and in clinical conditions resembling exophthalmic goiter, as tuberculosis, cardiac neuroses, etc., is a practical diagnostic application of Asher and Flack's original observations. The effect of the subcutaneous injection of adrenalin on the respiratory exchange in man was studied in 1912 by Fuchs and Roth (5), who found a slight increase in the oxygen intake and carbon dioxide output which they looked upon as negligible and an increase in the respiratory quotient. Later work by Bernstein (6), by Peabody and his co-workers (7), by Tompkins, Sturgis and Wearn (8) and by Sandiford (9) have clearly established that in normal men adrenalin injected subcutaneously in 0.5 cc. to 1.0 cc. (1–1000) doses causes an increase in the respiratory exchange. The studies of Tompkins, Sturgis and Wearn and of Sandiford are the more recent and extensive. The former, working with soldiers, were able to demonstrate the increase in twenty-seven of thirty-four cases. The latter studied forty-six cases including exophthalmic goiter, simple goiter, myxedema, Addison's disease and four normals. She concludes that the subcutaneous injection of 0.5 cc. 1–1000 adrenalin invariably causes an increase in the oxygen intake and the carbon dioxide output.

La Franca (10), working with dogs, found that phloridzin causes a marked decrease in the respiratory exchange, while adrenalin causes a marked increase both in the respiratory quotient and in the oxygen consumption. Hari (11) using curarized dogs and injecting adrenalin intraperitoneally observed a decrease in the oxygen consumption and a rise in the respiratory quotient. Lusk and Riche (12), studying the effect of adrenalin on the power of the animal to oxidize glucose, noted an increase in the respiratory exchange in two normal dogs. Wilenko (13) used urethanized rabbits and found no change in the respiratory quotient or the oxygen consumption.

In view of Asher and Flack's observation it has seemed to us of importance to compare the effects of the subcutaneous injection of adrenalin in normal and thyroidectomized animals and also to compare the effect on the same animal before and after thyroidectomy. The results of these experiments are given in the following pages.

Method. Rabbits have been used because it is possible to remove the thyroids without any physical interference with the function of the iind parathyroids and also because accessory thyroid tissue is less common than in dogs, cats or rats. We have used a Haldane (14) apparatus, modified by substituting Williams' absorbers and a motor-driven pump. This apparatus is readily adapted for the use of rabbits and is simple and accurate.

All rabbits were deprived of food for 15 to 16 hours before beginning the experiments. The adrenalin (P. D. Co., 1–1000) was not assayed. The dose was arbitrarily fixed at 0.5 cc. per kilogram. Each experiment consists of an hour period although in most instances the observations were repeated two or more times without interruption. This makes it possible to compare the results by hours or by longer units of time.

This study includes observations on six rabbits which had been kept in the laboratory for several months under the same conditions before being used in this work. Three rabbits (R 3–203, –205, –206) were "normal." Three had had thyroidectomies—one (3–208) 51 days before beginning the studies and two (3–201 and 3–204) during the studies. The detail figures obtained in each of these animals have been arranged in tables 2, 3, 4, 5, 6 and 7. For the brief discussion which follows, only the figures for the O_2 consumption per gram of body weight per hour will be considered. These have been averaged and arranged in table 1.

a. Controls (before administration of adrenalin). The average O_2 consumption for the five rabbits with intact thyroids was 0.607, 0.572, 0.524, 0.477 and 0.436 gram per gram of body weight per hour before the administration of adrenalin. These figures might be termed the basal rates and show the usual "normal" variations for different animals though the rate is relatively constant for a given animal. Rabbit 3–208 which had been thyroidectomized 51 days before beginning the observation shows the ordinary effect of thyroidectomy on metabolism first described in man by Magnus-Levy (16).

With rabbits 201 and 204 it is possible to compare the O_2 consumption before and after thyroidectomy. The figures given in table 1 represent the first series of observations before thyroidectomy and the last after thyroidectomy (30 and 31 days). The decrease in O_2 consumption following thyroidectomy is striking. Reference to tables 6 and 7 show that the decrease in the rate of metabolism following thyroidectomy is very slow—several days being required before the change becomes manifest. This may be due to the fact that the thyroid hormone is very stable and is normally needed in exceedingly small amounts.

TABLE 1

Average O_2 consumption per gram per hour in cubic centimeters

RABBIT NUMBER	CONDITION OF THYROID	CONTROL	FIRST HOUR AFTER 0.5 CC. ADRENALIN PER KILOGRAM	SECOND HOUR AFTER 0.5 CC. ADRENALIN PER KILOGRAM	THIRD HOUR AFTER 0.5 CC. ADRENALIN PER KILOGRAM	FOURTH HOUR AFTER 0.5 CC. ADRENALIN PER KILOGRAM	
203	Intact	0.607	0.637	0.697	0.789	0.680	0.708
205	Intact	0.436	0.528	0.549	0.595		
206	Intact	0.524	0.545	0.605			
208	Thyroidectomy 51 days	0.355	0.344	0.417	0.433	0.457	
204	Intact	0.477	0.651	0.610	0.530		
204	30 days after thyroidectomy	0.348	0.326	0.498	0.366		
201	Intact	0.572	0.530	0.681			
201	31 days after thyroidectomy	0.402	0.396	0.502	0.480		

These observations are in harmony with the clinical observations that the onset of symptoms of myxedema in thyroidectomized animals is slow and also that there is usually a latent period of 24 to 48 hours following the feeding of desiccated thyroid before an increase in the metabolic rate can be demonstrated. They are at variance with the effects on blood pressure as reported by Asher and Flack (loc. cit.) and by Levy (17). These authors showed that in acute experiments

TABLE 2

Rabbit 3–203. Thyroids intact

EXPERIMENT NUMBER	DATE	WEIGHT	DURATION	O_2 INTAKE	CO_2 OUTPUT	$\frac{CO_2}{O_2}$	O_2 PER GRAM PER HOUR	ADDITIONAL DATA
		grams		*grams*	*grams*		*cc.*	
1	5- 4-20	2125	3:20- 4:20 p.m.	1.570	2.210	1.02	0.516	Normal control
8	5- 6-20	2095	3:10- 4:10 p.m.	1.670	2.090	0.91	0.557	Normal control—1st hour
9	5- 6-20	2095	4:18- 5:18 p.m.	2.000	2.350	0.85	0.667	Normal control—2nd hour
19	5-11-20	2080	10:04-11:04 a.m.	2.170	1.990	0.67	0.729	Normal control—1st hour
20	5-11-20	2080	11:08-12:08 p.m.	2.170	2.090	0.70	0.740	Normal control—2nd hour
34	5-15-20	2025	10:36-11:36 a.m.	1.550	1.790	0.84	0.565	Normal control
36	5-15-20	2025	2:55- 3:55 p.m.	2.000	2.650	0.96	0.690	2:48 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram
42	5-18-20	2030	9:35-10:35 a.m.	1.620	1.950	0.87	0.558	Normal control—1st hour
43	5-18-20	2030	10:35-11:35 a.m.	1.650	1.680	0.73	0.568	Normal control—2nd hour
44	5-18-20	2025	1:15- 2:15 p.m.	1.860	2.440	0.95	0.642	1st hour following adrenalin injection. 1:05 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram
45	5-18-20	2025	2:15- 3:15 p.m.	2.330	2.720	0.85	0.804	2nd hour following adrenalin injection
46	5-18-20	2025	3:15- 4:15 p.m.	2.350	2.410	0.75	0.811	3rd hour following adrenalin injection
47	5-18-20	2025	4:15- 5:15 p.m.	1.970	2.300	0.85	0.680	4th hour following adrenalin injection
48	5-18-20	2025	5:15- 6:15 p.m.	2.050	2.140	0.76	0.708	5th hour following adrenalin injection
77	6- 4-20	2100	10:55-11:55 a.m.	1.710	1.870	0.79	0.569	Normal control
78	6- 4-20	2095	1:40- 2:40 p.m.	1.740	2.140	0.89	0.580	1.35 p.m. Injected subcutaneously 1.05 cc. adrenalin (P. D. Co. 1:1000) per kilogram. 1st hour following adrenalin injection
79	6- 4-20	2095	2:40- 3:40 p.m.	1.770	2.110	0.85	0.590	2nd hour following adrenalin injection
80	6- 4-20	2095	3:40- 4:40 p.m.	2.300	2.800	0.88	0.768	3rd hour following adrenalin injection

TABLE 3

Rabbit 3–205. Thyroids intact

EXPERIMENT NUMBER	DATE	WEIGHT	DURATION	O_2 INTAKE	CO_2 OUTPUT	$\frac{CO_2}{O_2}$	O_2 PER GRAM PER HOUR	ADDITIONAL DATA
		grams		*grams*	*grams*		*cc.*	
92	6–11–20	2180	9:30–10:30 a.m.	1.320	1.540	0.85	0.423	Control
93	6–11–20	2180	10:30–11:30 a.m.	1.330	1.370	0.75	0.426	Control
94	6–11–20	2170	2:50– 3:50 p.m.	1.750	2.400	0.99	0.564	2:42 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram. 1st hour following adrenal injection
104	6–21–20	2135	9:30–10:30 a.m.	1.300	1.390	0.78	0.426	Control
105	6–21–20	2135	10:30–11:30 a.m.	1.440	1.590	0.80	0.471	Control
106	6–21–20	2115	1:40– 2:40 p.m.	1.490	2.090	1.02	0.492	1:31 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram. 1st hour following adrenalin injection
107	6–21–20	2115	2:50– 3:50 p.m.	1.660	2.210	0.97	0.549	2nd hour following adrenalin injection
108	6–21–20	2115	4:00– 5:00 p.m.	1.800	2.060	0.83	0.595	3rd hour following adrenalin injection

TABLE 4

Rabbit 3-206. Thyroids intact

EXPERIMENT NUMBER	DATE	WEIGHT	DURATION	O_2 INTAKE	CO_2 OUTPUT	$\frac{CO_2}{O_2}$	O_2 PER GRAM PER HOUR	ADDITIONAL DATA
		grams		*grams*	*grams*		*cc.*	
81	6- 4-20	2390	9:45-10:45 a.m.	1.960	1.970	0.73	0.573	Control
82	6- 4-20	2390	10:45-11:45 a.m.	1.610	1.770	0.80	0.471	Control
84	6- 4-20	2380	2:20- 3:20 p.m.	2.050	2.350	0.83	0.602	1:15 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram 2nd hour following adrenalin injection
86	6- 4-20	2325	5:01- 6:01 p.m.	2.160	2.030	0.68	0.650	4th hour following adrenalin injection
100	6-19-20	2400	10:05-11:05 a.m.	1.710	2.090	0.89	0.498	Control
101	6-19-20	2400	11:05-12:05 p.m.	1.900	2.080	0.80	0.553	Control
102	6-19-20	2390	2:10- 3:10 p.m.	1.670	2.250	0.98	0.488	2:00 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram. 1st hour following adrenalin injection
103	6-19-20	2380	3:40- 4:40 p.m.	2.060	2.920	1.03	0.605	2nd hour following adrenalin injection

TABLE 5

Rabbit 3-208. R. and L. Thyroidectomy 3-22-20

EXPERIMENT NUMBER	DATE	WEIGHT	DURATION	O_2 INTAKE	CO_2 OUTPUT	$\frac{CO_2}{O_2}$	O_2 PER GRAM PER HOUR	ADDITIONAL DATA
		grams		*grams*	*grams*		*cc.*	
24	5-12-20	2445	11:53-12:53 p.m.	1.020	1.140	0.81	0.292	Control
25	5-12-20	2440	2:00- 3:00 p.m.	1.160	1.450	0.91	0.332	1:55 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram. 1st hour following adrenalin injection
26	5-12-20	2440	3:00- 4:00 p.m.	1.110	1.020	0.67	0.318	2nd hour following adrenalin injection
33	5-14-20	2410	4:35- 5:35 p.m.	1.450	1.590	0.80	0.421	Control
37	5-17-20	2500	10:21-11:21 a.m.	1.530	2.100	1.00	0.428	Control
38	5-17-20	2500	11:21-12:21 p.m.	1.400	1.990	1.03	0.392	Control
39	5-17-20	2495	1:43- 2:43 p.m.	1.390	2.020	1.06	0.393	1:33 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram. 1st hour following adrenalin injection
40	5-17-20	2495	2:43- 3:43 p.m.	1.510	2.160	1.04	0.423	2nd hour following adrenalin injection
56	5-20-20	2450	9:35-10:35 a.m.	1.320	1.580	0.87	0.377	Control
57	5-20-21	2450	10:35-11:35 a.m.	1.160	1.440	0.90	0.331	Control
58	5-20-20	2450	11:35-12:35 p.m.	1.040	1.350	0.94	0.297	Control
59	5-20-20	2445	2:00- 3:00 p.m.	1.220	1.510	0.90	0.349	1st hour following adrenalin injection. 1:51 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram
60	5-20-20	2445	3:00- 4:00 p.m.	1.580	1.650	0.76	0.452	2nd hour following adrenalin injection
61	5-20-20	2445	4:00- 5:00 p.m.	1.630	1.680	0.75	0.466	3rd hour following adrenalin injection
115	6-23-20	2530	9:40-10:40 a.m.	1.170	1.680	1.04	0.323	Control
116	6-23-20	2530	10:40-11:40 a.m.	1.220	1.540	0.92	0.337	Control
117	6-23-20	2525	1:20- 2:20 p.m.	1.090	1.590	1.06	0.302	1:16 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram. 1st hour following adrenalin injection
118	6-23-20	2525	2:20- 3:20 p.m.	1.720	2.090	0.88	0.476	2nd hour following adrenalin injection
119	6-23-20	2525	3:20- 4:20 p.m.	1.450	1.680	0.84	0.401	3rd hour following adrenalin injection
120	6-23-20	2525	4:20- 5:20 p.m.	1.650	1.600	0.73	0.457	4th hour following adrenalin injection

TABLE 6

Rabbit 3-204. Thyroidectomy 5-21-20

EXPERIMENT NUMBER	DATE	WEIGHT	DURATION	O_2 INTAKE	CO_2 OUTPUT	$\frac{CO_2}{O_2}$	O_2 PER GRAM PER HOUR	ADDITIONAL DATA
		grams		*grams*	*grams*		*cc.*	
49	5-19-20	2425	9:22-10:22 a.m.	1.710	1.960	0.83	0.493	Control
50	5-19-20	2425	10:22-11:22 a.m.	1.600	1.840	0.84	0.461	Control
52	5-19-20	2375	1:40- 2:40 p.m.	2.210	2.680	0.88	0.651	1:33 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram. 1st hour following adrenalin injection
53	5-19-20	2375	2:40- 3:40 p.m.	2.070	2.690	0.94	0.610	2nd hour following adrenalin injection
54	5-19-20	2375	3:40- 4:40 p.m.	1.800	2.240	0.90	0.530	3rd hour following adrenalin injection
66	5-24-20	2330	10:10-11:10 a.m.	1.500	1.750	0.85	0.450	Removed most of R. and L. thyroid lobes 5-21-20. Control
67	5-24-20	2330	11:10-12:10 a.m.	1.830	1.840	0.82	0.549	Control
68	5-24-20	2225	1:55- 2:55 p.m.	1.300	1.660	0.93	0.409	1:46 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram. 1st hour following adrenalin injection
69	5-24-20	2225	2:55- 3:55 p.m.	1.690	2.110	0.91	0.531	2nd hour following adrenalin injection
70	5-24-20	2225	3:55- 4:55 p.m.	2.030	2.010	0.72	0.638	3rd hour following adrenalin injection
71	6- 2-20	2400	9:20-10:20 a.m.	1.180	1.370	0.84	0.344	Control
73	6- 2-20	2400	1:50- 2:50 p.m.	1.220	1.860	1.11	0.355	1:15 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram. 1st hour following adrenalin injection
74	6- 2-20	2400	2:50- 3:50 p.m.	1.420	1.770	0.91	0.414	2nd hour following adrenalin injection
75	6- 2-20	2400	3:50- 4:50 p.m.	1.390	1.680	0.88	0.405	3rd hour following adrenalin injection
96	6-18-20	2490	10:55-11:55 a.m.	1.240	1.540	0.90	0.348	Control
97	6-18-20	2485	2:05- 3:05 p.m.	1.160	1.630	1.02	0.326	1:57 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram. 1st hour following adrenalin injection
98	6-18-20	2485	3:05- 4:05 p.m.	1.770	2.010	0.83	0.498	2nd hour following adrenalin injection
99	6-18-20	2485	4:05- 5:05 p.m.	1.300	1.710	0.91	0.366	3rd hour following adrenalin injection

TABLE 7

Rabbit 3-201. Thyroidectomy 5-21-20

EXPERIMENT NUMBER	DATE	WEIGHT	DURATION	O_2 INTAKE	CO_2 OUTPUT	$\frac{CO_2}{O_2}$	O_2 PER GRAM PER HOUR	ADDITIONAL DATA
		grams		*grams*	*grams*		*cc.*	
62	5-22-20	2360	3:15- 4:15 p.m.	1.960	2.000	0.74	0.581	Control
63	5-22-20	2360	4:15- 5:15 p.m.	1.900	1.830	0.70	0.563	Control
64	5-22-20	2360	5:30- 6:30 p.m.	1.790	2.530	1.03	0.530	5:19 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram; 1st hour following adrenalin injection
65	5-22-20	2360	6:30- 7:30 p.m.	2.300	2.320	0.73	0.681	2nd hour following adrenalin injection
87	6-10-20	2840	9:20-10:20 a.m.	1.460	1.950	0.97	0.359	Control
88	6-10-20	2840	10:20-11:20 a.m.	1.170	1.480	0.92	0.295	Control
89	6-10-20	2825	1:50- 2:50 p.m.	1.340	2.290	1.33	0.332	1:45 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram urinated in chamber. 1st hour following adrenalin injection
91	6-10-20	2825	4:15- 5:15 p.m.	2.020	2.230	0.81	0.513	3rd hour following adrenalin injection
109	6-22-20	2860	9:20-10:20 a.m.	1.750	2.230	0.93	0.428	Control
110	6-22-20	2860	10:20-11:20 a.m.	1.550	1.850	0.87	0.386	Control
111	6-22-20	2860	11:35-12:35 p.m.	1.610	2.130	1.10	0.394	Control
112	6-22-20	2840	2:00- 3:00 p.m.	1.610	2.470	1.11	0.396	1:54 p.m. Injected subcutaneously 0.5 cc. adrenalin (P. D. Co. 1:1000) per kilogram. 1st hour following adrenalin injection
113	6-22-20	2840	3:10- 4:10 p.m.	2.040	2.650	0.94	0.502	2nd hour following adrenalin injection
114	6-22-20	2840	4:21- 5:21 p.m.	1.950	2.300	0.86	0.480	3rd hour following adrenalin injection

lasting only a few hours the effect of adrenalin on blood pressure was markedly decreased by thyroidectomy. The cause of these time differences between the effect of adrenalin on blood pressure and on metabolism in animals with and without thyroidectomy is not clear.

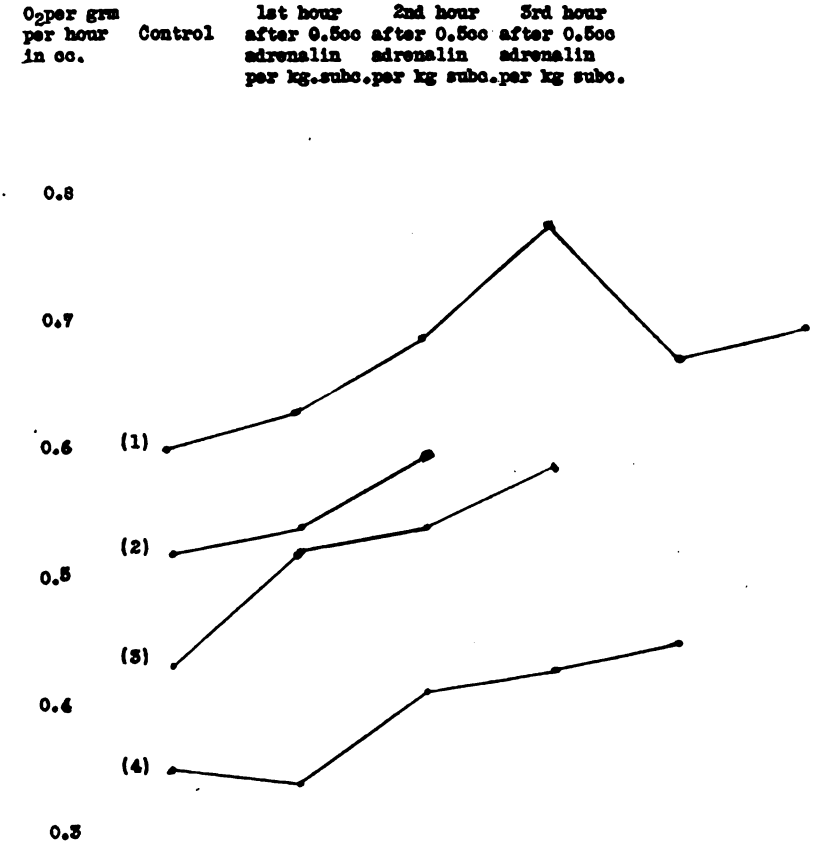

Fig. 1. Average O_2 consumption per gram per hour in cubic centimeters. *1*, Rabbit 203, thyroids intact; *2*, rabbit 206, thyroids intact; *3*, rabbit 205, thyroids intact; *4*, rabbit 208, thyroids removed (51 days).

b. After adrenalin administration. In each case 0.5 cc. per kgm. (P. D. adrenalin 1–1000) unassayed stock adrenalin was injected subcutaneously in the flank. The observations were recorded in hourly periods following the injection. In all instances a rise in the O_2 consumption was noted. The changes are shown graphically in text figures 1, 2 and 3. It occurs as well in the thyroidectomized animals as in "normals." This is brought out best in figures 2 and 3 when the effect of adrenalin before and after thyroidectomy may be compared in

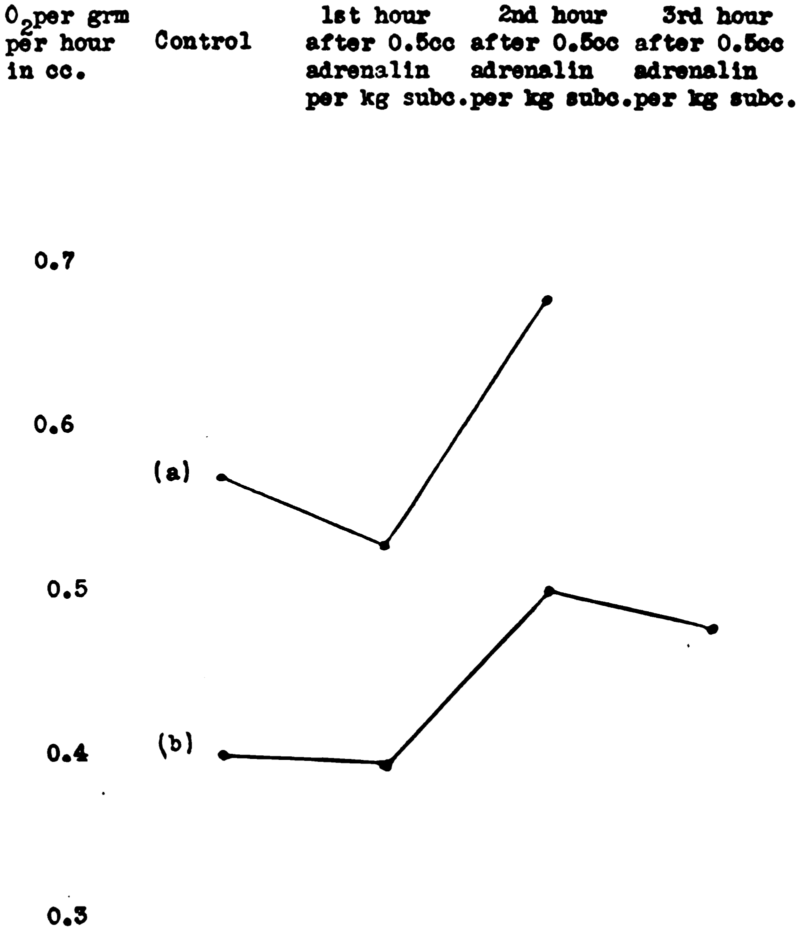

Fig. 2. Rabbit 201. Average O_2 consumption per gram per hour in cubic centimeters. *a*, Thyroids intact; *b*, thyroids removed (31 days).

the same animal. The percentile rise in O_2 consumption appears to be little changed by thyroidectomy. These results confirm Sandiford's observations in human myxedema. The adrenalin effect lasts for hours. We have observed it for five hours, though usually the greatest increase is reached in the third hour. There is some evidence that in the thyroidectomized animals the onset of the increased rate of metabolism is delayed and also the reaction is of shorter duration as

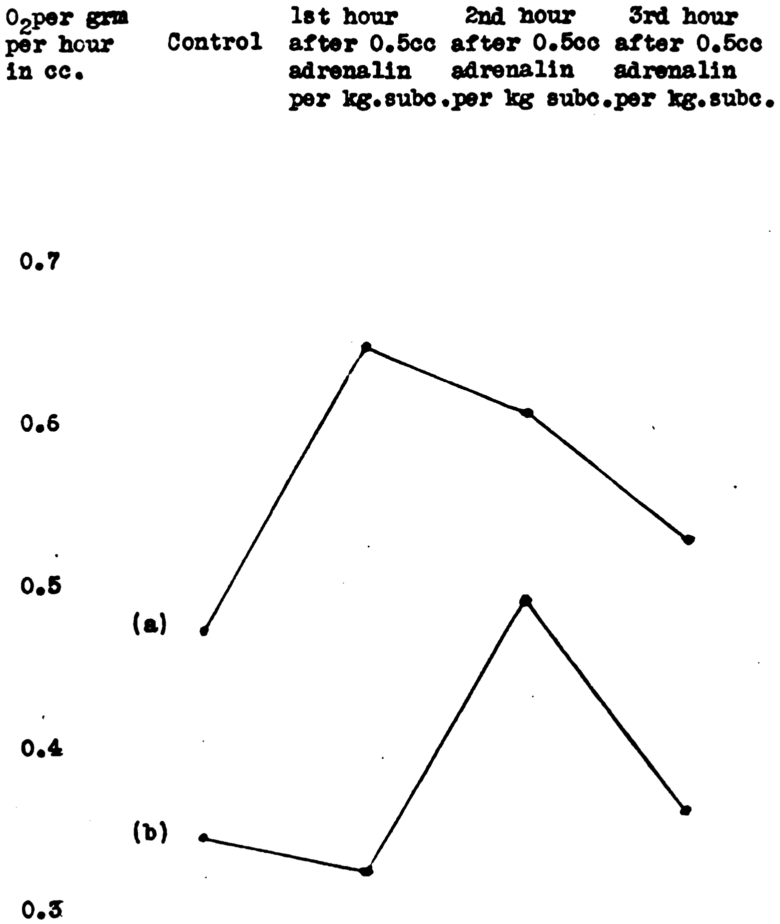

Fig. 3. Rabbit 204. Average O_2 consumption per gram per hour in cubic centimeters. *a*, Thyroids intact; *b*, thyroids removed (30 days).

shown in figures 2 and 3. Another observation on the effect of adrenalin in thyroidectomized animals is that frequently there is a decrease in O_2 consumption during the first hour after adrenalin. This was also observed in "normals" but very rarely.

SUMMARY

Our results on rabbits confirm those of Sandiford on man. Adrenalin causes a rise in the oxygen consumption both in normal and thyroidectomized rabbits. The absolute rise may be greater in normals but the percentile rise may not be altered. Evidence is given that in general the onset of the rise in O_2 consumption following adrenalin is delayed in thyroidectomized animals and also that it does not last so long. Some evidence is presented showing that in rabbits as in other animals the decrease in the metabolic rate following thyroidectomy is gradual and requires several days for its demonstration. These results differ from those in which the effect on blood pressure was used as the indicator.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

III. Lung Automatism and Lung Reflexes in Reptilia (Turtles: Chrysemys elegans and Malacoclemmys lesueurii. Snake: Eutenia elegans)

A. J. CARLSON and A. B. LUCKHARDT

From the Hull Physiological Laboratory of the University of Chicago

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LITERATURE

The first observations on the contractility of the reptilian lung on direct stimulation of the pulmonary tissue was made by Paul Bert (1). He showed furthermore that the musculature of the lung was under the motor control of the vagus nerve. These findings have been abundantly corroborated by the subsequent researches of various investigators of whom François-Franck (2) deserves particular mention.[1] Francois-Franck (3), (4) published a number of papers dealing with the comparative physiology of the reptilian lung. The results of these studies are incorporated in two monographs which as far as they touch our work are the most comprehensive and important contributions on the subject. In most forms the vagus is found to exercise a motor control over the lung of the same side. In one lizard (lézard ocelle) the lungs possess in part a crossed innervation the vagus exercising not only a homolateral but also a contralateral control over the lungs. François-Franck (5) states that the vagi and not the sympathetic nerves contain the motor fibers for the lungs. Jackson and Pelz (7), on the other hand, state that stimulation of the sympathetic in the neck region with a weak current causes dilatation of the lung and that stronger currents may give a contraction even more promptly and vigorously than stimulation of the vagus itself. In some preparations François-Franck (4), (5) found that electrical stimulation of the vagus in the neck gave rise to a contraction which was preceded by an inhibition. According to his experimental analysis this inhibition is only apparent. He attributes it to a movement of neck muscles. If the muscles of the neck were not involved during peripheral stimulation of the vagus no inhibition preceded the contractions. Personally, we have never obtained an inhibition of the lung on direct stimulation of the peripheral vagus. We have, however, in innumerable instances observed an inhibition preceding lung contractions of central origin. We shall return to this point later in a discussion of this phenomenon.

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[1] Some 32 years after the original observations of Bert, Maar (Skand. Arch. f. Physiol., 1902, xiii, 269) published an article on the gaseous exchange in the lungs of turtles following ligation and stimulation of the vagi and sympathetic nerves under various experimental conditions. This author apparently was not familiar with the fact that the lungs of the turtle are muscular organs innervated through motor fibers carried by the vagus. The possibility that changes in gaseous exchange (increased oxygen consumption and increased CO_2 following ligation of the vagus) might be accounted for by contraction of the lung (mechanical stimulation of motor fibers) never occurred to this investigator. Practically all positive findings which he attributes to section and stimulation of fibers having a secretory or inhibitory effect on gaseous exchange might be explained by the activity or inactivity of the lung musculature and the passive influence of the circulation through the lungs.

Kahn (8) and François-Franck (5) have described and pictured rhythmical contractions of the lung due to central discharges from the bulbar nuclei; for they stopped on section of the vagi. Kahn in his analysis on turtles which had suffered high spinal transection showed that these lung contractions followed the external respiratory act; François-Franck noted these rhythmical undulations in the intrapulmonic pressure even in curarized animals but only when the bulbar centers and the vagi were intact. He was never able to obtain a tonus rhythm in any lung separated from its center. He found on electrical stimulation of the peripheral end of the vagus that a secondary contraction may appear on the completion of first; or if the peripheral vagus was tetanized for some time a series of contractions might appear during the period of tetanization simulating tonus variation of the lung. In both instances, however, he was probably dealing with incomplete tetany of the lung rather than with discharges from a peripheral nervous automatic mechanism as a result of the vagus stimulation.

A tonus rhythm appearing in a lung whose extrinsic nerves have been cut would suggest a peripheral nervous mechanism possessing automatic activity. On the anatomical side we have the observations of Leydig (9) and Schulze (10) that the lungs of some turtles possess not only ganglionic swellings on the course of the nerve fibers but actual accumulations of ganglion cells. On the physiological side Fano and Fasola (2) have asserted that the oscillations of tonicity in the lungs of the turtle, Emys europaea, persist after complete isolation of the lungs from their nervous center although they admit that under normal conditions part of the tonicity is due to impulses reaching the lung through their extrinsic nerves. Since such a peripheral automatism of the lung did not reveal itself in the turtle studied by François-Franck (tortue grecque) and only occasionally in our own preparations it would seem that the question of a peripheral automatic mechanism in the lung may depend on the species as well as on the physiological condition of the animal under observation.

The published reports of lung contractions as a result of the stimulation of various afferent nerves in the reptilia are very meager. Certainly François-Franck, who made a most extensive study of the physiology of the reptilian lung, refers only incidentally to rhythmical lung contractions set up by stimulation of afferent fibers carried by the vagi (3). In his second monograph (4) on the lizard (lézard ocelle) he describes a similar contraction of one lung following ligation of the vagus of the opposite side (mechanical stimulation). Since in this preparation there was intercommunication between the lungs and since he showed that in this species of reptiles the vagus contained motor fibers not only for the lung of the same side but also for the lung of the opposite side, it is possible to explain his supposed reflex contraction on ligation of the vagus nerve to direct motor effects on the opposite lung or to the passive distention of the lung of the opposite side of ligation of the vagus. Both factors would give a record which might be interpreted as an active reflex contraction of the opposite lung.

Prevost and Saloz (10) are the only investigators who, by a method inferior to our own, have described reflex contractions of the lungs of the turtle (tortue grecque). They found that trauma to the carapace, mechanical stimulation of feet, tail, neck and anal region, caused marked contractions of the lungs.

Coombs (12) has recently shown that stimulation of the optic lobes or the medulla in the turtle induced contractions in the lung, provided the vagi are intact. This seems to indicate that the motor innervation to the lungs passes exclusively by the vagi nerves, unless the sympathetic nerves were sectioned by her method of lung isolation.

EXPERIMENTAL PROCEDURE

The solution of the problems under investigation required an accurate recording of the lung tonus and lung contractions with the least possible injury to or interference with the normal respiration and other processes of the animal. In other words, the necessary aim and requirement so far as possible was to secure *physiological experiments.*

1. Preparation of the animal. No anesthetics were used. Prior (1 to 24 hours) to any dissection or other procedures that would cause pain, the animals were decerebrated by quickly drilling through the skull a little to the side of the median line, introducing a suitable probe through this opening and after cutting off the cerebrum from the midbrain by the transverse stroke virtually pithing the former. By using a small drill and going 2 to 4 mm. lateral to the median line, the median sinus is left intact and profuse hemorrhage avoided. By our method of pithing the cerebrum very little hemorrhage was produced, so that from this factor alone there was little or no impairment to the general circulation or interference with the blood supply of the medulla and midbrain. A small iron hook was introduced into the trephine hole in the skull, by means of which the head was secured to the post as shown in figure 1. The hook being placed in an insensitive spot, the head and neck were thus fixed without the complications from continued irritation of clamps or ligatures about head and neck.

By means of a small drill, holes were made near the edge of the plastron anteriorly and posteriorly, and by strings through these holes in the ventral plate the animal was secured on the turtle stand in the manner shown in figure 1. In the species of turtles worked on, there is a sufficient margin of the ventral plate at both ends for drilling the hole and securing the animal on the stand in its normal posture without touching or injuring the skin.

A few animals were worked on without decerebration. In this case the spinal cord was transected in the neck before any operations were undertaken on the body of the animal.

For convenience of dissection the turtle stand with the animal attached was turned upside down and held in that position by a suitable support while the trachea, the carotids, the jugular vein, the cervical sympathetics, etc., were isolated, balloons introduced into the stomach (via esophagus) and the cannulae and other devices placed in position in the neck and anterior thoracic region.

Isolation of the lungs and other viscera was made by removal of the necessary regions of the dorsal carapace. Cutting through and removing parts of the dorsal carapace is attended with considerable hemorrhage unless care is taken not to injure the intercostal arteries and veins when the calcareous part of the body wall is sawed through or clipped off with strong bone forceps. If suitable care is exercised in this regard, the diploic veins controlled by surgical wax and the intercostal vessels ligated before cutting away the peritoneum and other structures that adhere closely to the calcareous part, the entire body cavity can be laid open from the dorsal side, with very little immediate and with no chronic hemorrhage.

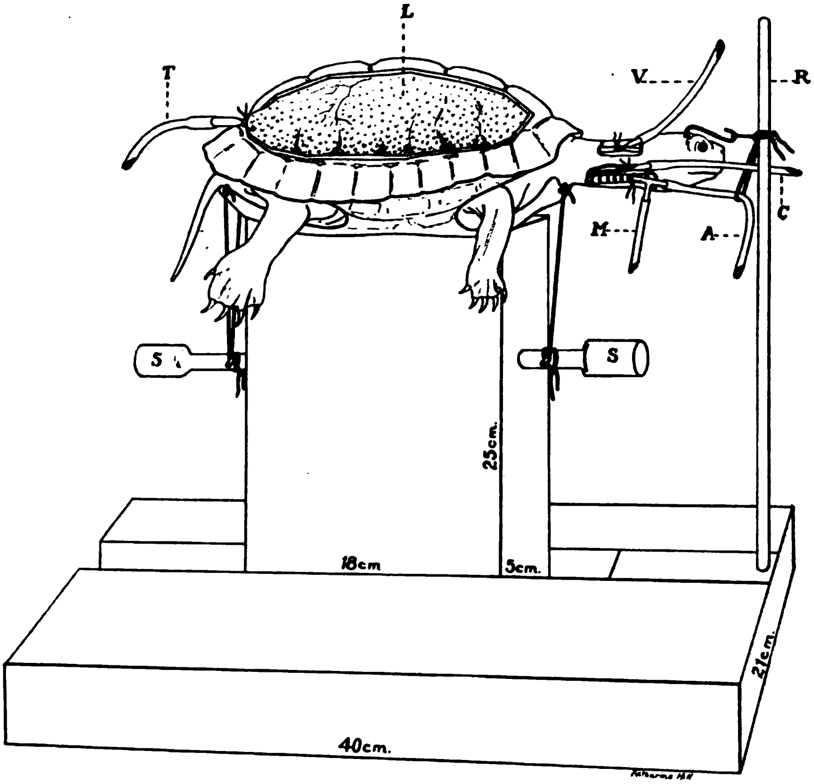

Fig. 1. Diagram of preparation and fixation of the turtle for recording lung contractions. *A:* tube to T-tracheal cannula for spontaneous or artificial respiration in the intact lung. *C:* Cannula in the carotid artery for recording the blood pressure and heart activity. *L:* Lung, isolated, except for pulmonary vagi and blood vessels, bronchus ligated off. Inflated in its normal position in the body cavity. *M:* Tube to manometer for recording the spontaneous respirations. *R:* Flexible steel rod for fixation of the head. *S:* Screws for the fixation of turtle to the top of stand by strings through holes in the plastron. *T:* Cannula and rubber tubing from tip of isolated lung to recording tambour. *V:* Cannula in the jugular vein for intravenous injections.

In a few experiments where it was desired to avoid the injury to visceral sensory nerve connections involved in isolating one lung from the dorsal side, the lungs were exposed and isolated by fixing the animal dorsal side down, removing the entire plastron and dissecting away the liver and greater part of the gut in the way described by Jackson and Pelz (7). In animals fixed on the dorsal side and plastron removed the circulation fails much sooner than if the animal is fixed in the normal position and the viscera exposed by removing one side of the dorsal carapace.

In all our preparations exposing the lungs and viscera from the dorsal side, the dorsal carapace was left intact along the vertebral column, a strip 1 to 1½ cm. wide. This precaution, together with reasonable care in isolating the lung from its attachments along the median line, leaves the main central connections of the visceral sympathetic nerves, except possible fibers to the lung, intact.

2. Isolation of the lung. As is well known, the lung of the turtle is a bilobed organ, united by the two long (3 to 5 cm.) bronchi with the very elongated trachea. The lungs are exceptionally large in comparison with the size of the animal, filling the dorso-lateral region down to the pseudo-diaphragm separating the pelvic cavity and the urinary bladder from the rest of the viscera. Except for the posterior end (about one-sixth of the entire area) the lungs are so firmly attached by fibrous septa and membranes, dorso-laterally to the carapace, and ventro-medially to the visceral organs, that complete collapse of the lungs is impossible without severing the lungs from these connections. It is therefore obvious that accurate recording of lung tonus and lung contractions requires either complete anatomical isolation of the lung from structures whose contractions could influence the lung volume, directly or indirectly, or else complete curarization of the animal. We used both procedures.

Complete isolation of one lung, usually the left, was produced in the following way. The animal being decerebrated, fixed on the turtle stand, as described above, the dorsal carapace over the lung was removed, all the septa and membranes suspending the lung in the body cavity were severed, care being taken not to injure the bronchus, or the pulmonary vagi and blood vessels entering the lungs along the bronchus. In this dissection the lung was not handled directly with forceps or other instruments. It was handled by means of the many membranes and tendons attached to it. It is not necessary to remove these structures close to the lung surface since they can have no effect on the lung tonus and lung contractions after being severed from their normal connections with the rest of the body. This applies even to the large flattened striated muscle closely adherent and attached to the anterior end of the lung. Directed median- and antero-laterally over the dorsal lung wall and being innervated by fibers from the brachial nerve plexus, the contraction of this muscle reduces the size of the lung cavity and thus serves as a muscle of expiration (fig. 24), supplementing the expiratory muscles of the flank and the walls of the body cavity. This muscle is probably identical with the one described by Fano and Fasola as occurring in Emys europaea (2).

This operation and isolation of the left lung does not cause collapse of the right lung or vice versa. There is naturally some interference with the adequate ventilation in the lung of the intact side by the lowering of the intra-abdominal pressure and the incapacitation of the respiratory flank muscles on the side of the operation, but the animal is usually capable of filling and emptying the lung on the intact side to meet the respiratory needs of the body, especially if one takes care not to puncture the delicate septa partially separating the left and the right side body cavities along the line of the great retractor muscle of the neck.

In figure 2 are shown by diagram the main vago-sympathetic nerve connections. The pulmonary vagi branches, usually two or more in number, are of sufficient size and length to be easily handled for experimental purposes; and the bronchus may be ligated and cannulated without injury to the pulmonary nerves or vessels, and the pulmonary vessels may be ligated or cannulated without injury to the nerves.

There is a persistent tendency on the part of some anatomists and zoölogists, as well as physiologists, to speak of the "bronchioles" or bronchial musculature of the turtle's lung. This is entirely misleading. In species of turtles worked on by us the bronchus terminates peripherally, not by the mode of branching into smaller and smaller divisions (bronchioles), but abruptly in the general lung cavity. We are told by the anatomists that the numerous septa subdividing the lung cavity into irregular chambers have strands of smooth muscle fibers like the external walls of the lungs. But the smallest passages between these chambers are of larger diameter than the trachea itself. These passages and septa are alveolated. If these internal septa and irregular passages are to be termed "bronchioles" on the basis of their musculature, the entire lung of the turtle is composed of "bronchioles." These subdivisions in the turtle lungs are alveolar spaces and not part of the dead space similar to the mammalian bronchioles.

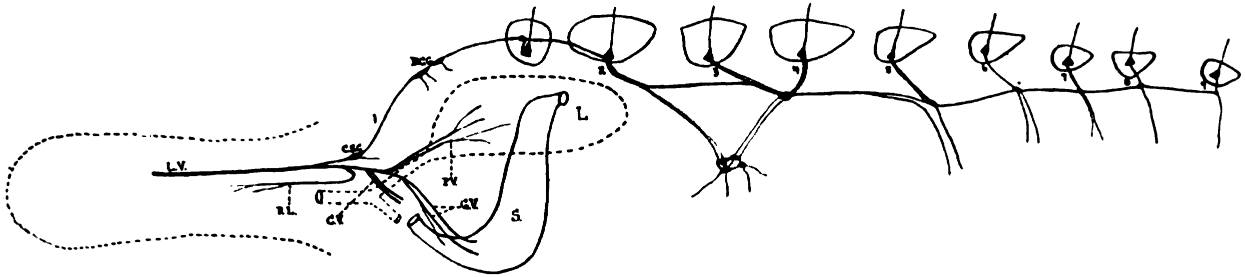

Fig. 2. Diagram of the vago-sympathetic nervous system (left side, ventral view) of the turtle. The diagram of the sympathetic system is not completed posteriorly (sacral region). *L:* Left lung (greatly reduced). *S:* Stomach. *L.V.:* Left vagus. *R.L.:* Recurrent laryngeal nerve. *C.V.:* Cardiac vagi branches. *G.V.:* Gastric vagi branches. *P.V.:* Pulmonary vagi branches. *C.S.G.:* Cervical sympathetic ganglion. *B.S.G.:* Sympathetic ganglia in close association with the brachial nerve plexus. *1:* Cervical sympathetic nerve. *2* to *9:* Nerve connections from the spinal ganglia to the peripheral sympathetic ganglia and nerve plexuses.

3. Recording of the lung contractions. The lung contractions were recorded graphically by water manometers or delicate tambours through air transmission in either case, suitable cannulae being tied either into the bronchus or the free end (posterior tip) of the isolated lung, the isolated lung being distended with air to a pressure of 1½ to 3 cm. of water. In such a preparation active voluntary respiration in the lung on the intact side will still cause passive changes in the intrapulmonic pressure in the isolated lung through the movements of the viscera (on which the isolated and inflated lung rests) especially as a result of very vigorous expiratory movements. We eliminated this source of error in two ways. The isolated and inflated lung was suspended outside the body cavity without traction on the pulmonary nerves or interference with the lung circulation. Or a metal plate was adjusted to the body cavity on the operated side, fixed rigidly to the cut edge of the carapace, except anteriorly, and the isolated and inflated lung placed on this rigid floor, where visceral movements could not influence it. This method can be adjusted so as not to cause traction on the pulmonary nerves, or interference with the pulmonary circulation. The best, and possibly the most physiological method of eliminating these passive lung factors, is partial curarization, that is, doses of curare that will completely paralyze the skeletal muscles, but leave the muscles of respiration largely intact. In our animals this dose varied from ⅛ to ¼ cc. of 1 per cent solution of curare injected intravenously. Turtles subjected to these doses of curare go on breathing regularly though not vigorously, leg and neck movements are abolished, and the gentle respiratory movements on the intact side either do not mechanically influence the isolated and inflated lung on the operated side at all, or else this passive influence is regular or so slight that it is no source of error in the work.

When all these dissections were completed and connections for recording made, the dorsal opening in the carapace was covered with a thick layer of cotton moistened in Ringer's solution, except during such phases of the work as required direct inspection of or working with the isolated lung or its local nerve and blood supply.

4. Artificial respiration. In all cases of complete curarization, artificial respiration was carried out periodically, usually in the intact lung. For that purpose a T-cannula was usually inserted in the trachea. This tracheal cannula was also used in studying the reflex influences of pressure changes in the intact on the isolated lung. The venosity of the blood in the isolated lung was a satisfactory criterion of the need of artificial respiration. Needless to say, artificial respiration was also resorted to in the animals not curarized, if it appeared that the spontaneous respiration through the lung on the intact side did not suffice to avert asphyxia.

It is well known that the reptilia execute the same buccal respiratory movements as the amphibia, although actual swallowing of the air into the lungs does not occur. However, in labored respiration as in mild or severe asphyxia, the reptilia carry on actual swallowing movements as a part of the respiratory act. These swallowing movements may thus be used as an index of external respiration, or rather attempt at external respiration, in animals with the respiratory muscles immobilized by transection or pithing of the spinal cord.

5. Maintenance of efficient circulation. If we start with an animal vigorous and in good condition, and care is taken to avoid all but the minimum hemorrhage in the experimental procedures, a good circulation will be maintained as shown by direct blood pressure records or by direct inspection of the isolated lung, for 12 to 24 hours or even longer. In feeble animals the circulation fails much earlier. The circulatory failure is due, not to the failure of the heart, but to low blood pressure evidently due to *transudation of the blood plasma into the lymph and tissue spaces.* This is not ordinary oozing of blood from cut surfaces or hemorrhage due to injured vessels. In a turtle preparation in this condition intravenous injection of Ringer's solution improves the circulation only temporarily, the added fluid soon passes out of the blood vessels into the lymph and tissue spaces, and repeated Ringer's solution injections then render the preparation more edematous.

It is not unlikely that this phenomenon is analogous to the passage of plasma from the blood to the tissues in traumatic shock in mammals, as reported by some investigators.

6. Additional preparations of the animal for studying some of the lung reflexes. The turtle preparation described above requires no additional dissection for the study of the lung reflexes induced by inflation or deflation of the intact lung, stimulation of the nares, the cloaca, the penis, the skin or the skeletal nerves. It is even possible by careful manipulation to get at and stimulate the bladder, the rectum, ureter, large and small intestine, etc., through the opening in the dorsal carapace made for the isolation of the lung, without altering the tension on the lung wall mechanically. In the artificial stimulation of the central end of the pulmonary, gastric and vagi branches on the side opposite to the lung under observation it is usually necessary to make a small opening in the dorsal carapace on that side and partially collapse the intact lung. In the same way inflation of the urinary bladder or the rectum, direct stimulation of the gall bladder, stomach, etc., requires an opening of the carapace posteriorly on the side opposite to the lung under observation, if one is to avoid mechanical errors.

7. On the basis of our own results, and with due regard to methods used by previous investigators on various phases of reptilian respiratory physiology, we feel that a turtle prepared as above with minimum trauma, fixed in normal position without trauma, one lung isolated for accurate observation, the other intact and used in normal spontaneous respiration, is as near its normal physiological state as the necessities of the problem permit. Most phases of lung physiology in the turtle cannot be satisfactorily studied with the animal fixed with the dorsal side down, owing to the special anatomical conditions.

The serious and unavoidable difficulties, apart from that of a laboratory located in a city obtaining turtles in prime condition, are *a*, depressor effects or "central shock" due to sensory effects involved in the operative trauma; *b*, "peripheral shock" or gradual failure of the circulation due to passage of the blood plasma out of the blood vessels into lymph and tissue spaces. It is generally held that reptiles show little or no spinal or traumatic shock, and this was our main reason, besides that of avoiding anesthesia, for working out the visceral reflexes first in this form. Shock, spinal and general, may be minimal but is certainly not absent in the turtle.

8. *Experimental procedure on the snake.* No satisfactory work on the contraction of the lung in these animals can be done without virtually removing the lung from the body, complete pithing of the spinal cord, or complete curarization, thus immobilizing the body. Curarization is, of course, not applicable in experiments on the relation of the lung contractions to the external respiratory act. Hence we used the method of complete pithing of the spinal cord after transection of the cord two or three segments below the medulla. The animal was then fixed, dorsal side down, the lung exposed and isolated by a ventral median incision, taking care to avoid hemorrhage. The recording cannula was inserted in the tip of the lung rather than in the trachea. The trachea was ligated after isolation from the vagi and the neck blood vessels. This preparation is suited only for studying the relation of the respiratory movements to the lung contractions, the vagi action on the lungs, and the lung reflexes evoked from the head region of the animal. In the pithed snake the external respiratory movements appear in the form of attempts at swallowing air just as in the turtle.

In the species of snake used by us the lung is not a paired organ. Alveoli and septa are present in the anterior third of the lung only, the posterior two-thirds of the lung being a delicate apparently muscular sac very much like the primitive lung of necturus. There being only one lung, the necessary artificial respiration had to be carried out periodically in the lung while suspending the recording. This fact renders the snake much less suitable than the turtle for the study of lung motor physiology.

The musculature of the snake lung is very much less developed than that of the turtle. Even delicate water manometers are not sufficiently sensitive for recording the lung contractions. The snake preparations also deteriorate very much faster than does the turtle. Because of these several handicaps the work on the snake was only carried to the point of establishing the identity of the fundamental facts of lung motor physiology in these two groups.

THE RHYTHMIC CONTRACTIONS OF THE LUNGS DURING NORMAL RESPIRATION AND AFTER PARTIAL AND COMPLETE CURARIZATION

1. Typical records of the active lung contractions that follow each external respiratory act or a group of respirations are reproduced in

Fig. 3. Water manometer tracings of the intrapulmonic pressure in the turtle. Animals decerebrated. *A:* Record from left lung; dorsal shell removed on left side, lung isolated except nerve and blood vessel connections. Cannula in left bronchus. The spontaneous respiratory movements at *a* passively influencing the isolated lung. Each group of respiratory movements is followed by contraction of the isolated lung. *B:* Tracing from the same animal as in *A*, 24 hours later after recovery from curare. Record from the intact (right) lung. Cannula in trachea. Each group of respirations is followed by contraction of the lung. *C:* Tracing from left lung, isolated as in *A*, after intravenous injection of a dose of curare (¼ cc. of 1 per cent, to paralyze skeletal muscles). Signal equals gasping or swallowing movements. These are followed by lung contractions. *D: c*, left lung isolated as in *A*; *b*, right lung in normal attachments. *a*, carotid blood pressure (Hg.); signal equals respiratory movements; ¼ cc. curare injected intravenously leaving slight respiratory movements of mouth and flanks. Showing lung contractions following respiratory movements. *E* and *F:* Spontaneous contraction of lungs after a large dose of curare (½ cc. 1 per cent) completely paralyzing all striated muscles. *E*, lung isolated as in *A*. *F*, lung in normal attachment. Showing rhythmic lung contractions in the absence of external evidence of respiration. *G* and *H:* Water manometer records from inflated balloon in the stomach of the turtle; shell intact; stomach atonic and quiescent, but showing beginning of feeble rhythm at *c; a*, spontaneous respiratory movements, quick up and down stroke, followed by lung contractions; *b*, shown as diminished tension in the stomach because of the lowered intra-abdominal pressure created by the active lung contraction, the shell being intact. Time, 5 seconds.

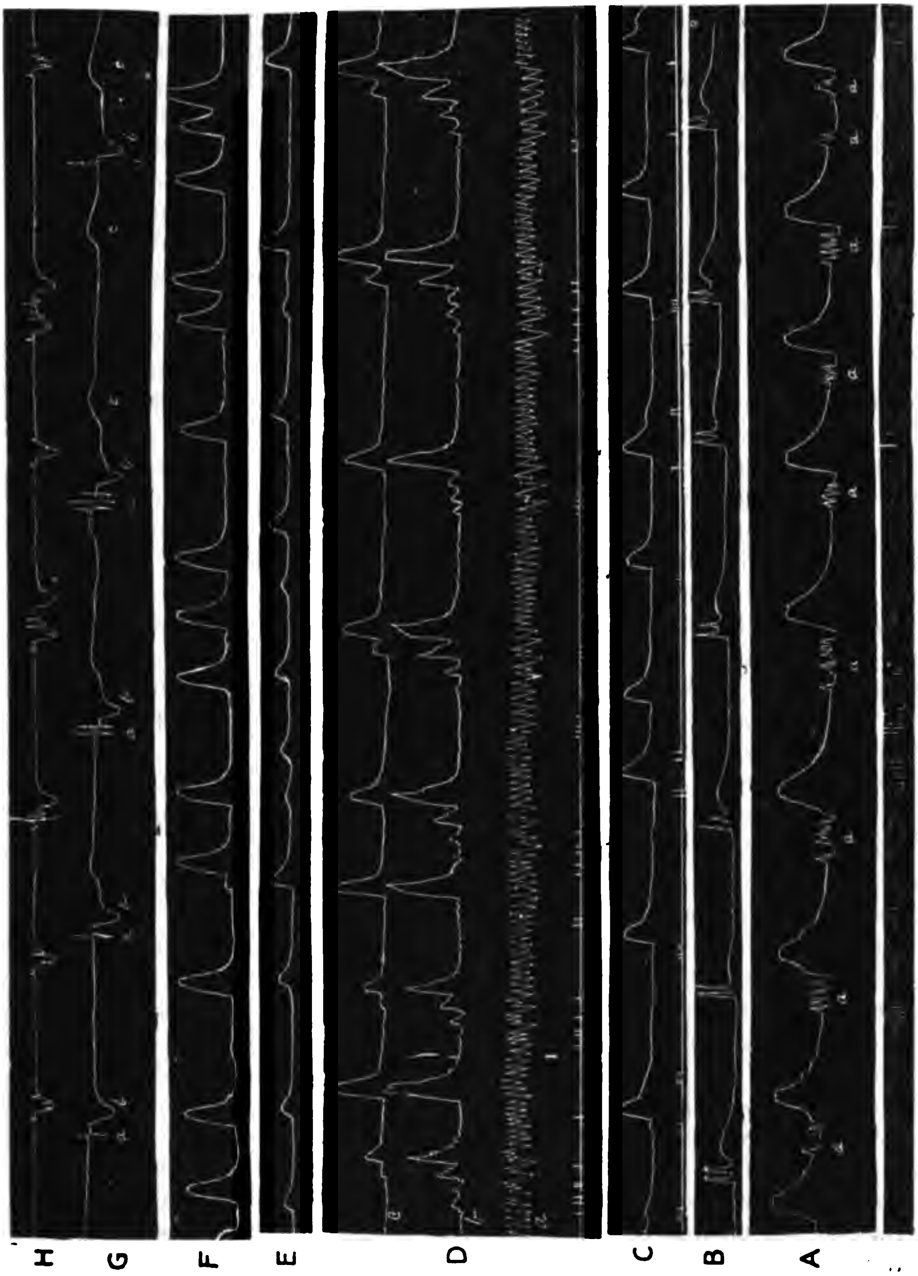

Fig. 3

figure 3, *A*, *B*, *C*. The lung contraction follows the respiration after a latent period of 1 to 3 seconds. In preparations in poor condition the latent period is usually much longer. If the external respiratory movements are of the Cheyne-Stokes type, the lung contraction does not appear until the end of the last respiration in the group, especially if the respirations come fairly close together, except in marked asphyxia. The lung contractions are usually absent when the external respiration is rapid and continuous. This absence appears to be due to inhibition of the center controlling the lung contractions by some process in the external respiratory act.

Under certain conditions acts of external respiration may be executed without being followed by a contraction of the lung. This is especially the case in animals in poor condition from any cause, in preparations in partial traumatic shock from the operative procedure, or if for any reason, such as apnea, the external respiration is feeble. By feeble we mean feeble discharges from the respiratory center. The general relation obtains that the stronger external respiratory act, the stronger the lung contractions that develop during the respiratory pause.

Graphic records of this strong lung rhythm may be obtained from an inflated balloon in the stomach of the turtle, provided the stomach is relatively quiescent and in low tonus (fig. 3, *G* and *H*). The external respiratory act induces passive changes in the intragastric pressure (fig. 3, *G*, *a*) just as in mammals, except reverse, that is, in the turtle expiration causes increased intragastric pressure and vice versa. The active lung contractions appear on the gastric balloon records as waves of negative pressure or lowered tonus, owing to the lung contraction increasing the general intra-abdominal negative pressure.

Tracings showing the lung rhythm as it appears after complete curarization are reproduced in figure 3, *E* and *F*. If a paralyzing dose of curare is injected intravenously in a preparation breathing spontaneously and regularly and exhibiting lung contractions as in figure 3, *A*, the brief central stimulation phase of the curare action, as shown by violent body movements and cardiac arhythmia, is followed by complete quiescence of the lungs lasting for one-half to two hours. This is not due to peripheral paralysis of the pulmonary motor nerve fibers. It is evidently due to paralysis of the medullary centers by curare. Reflex lung contractions can be secured before the spontaneous rhythm appears. But when the spontaneous lung rhythm reappears, it continues for hours or indefinitely provided suitable artificial respiration is carried out at intervals and a fairly efficient circulation maintained.

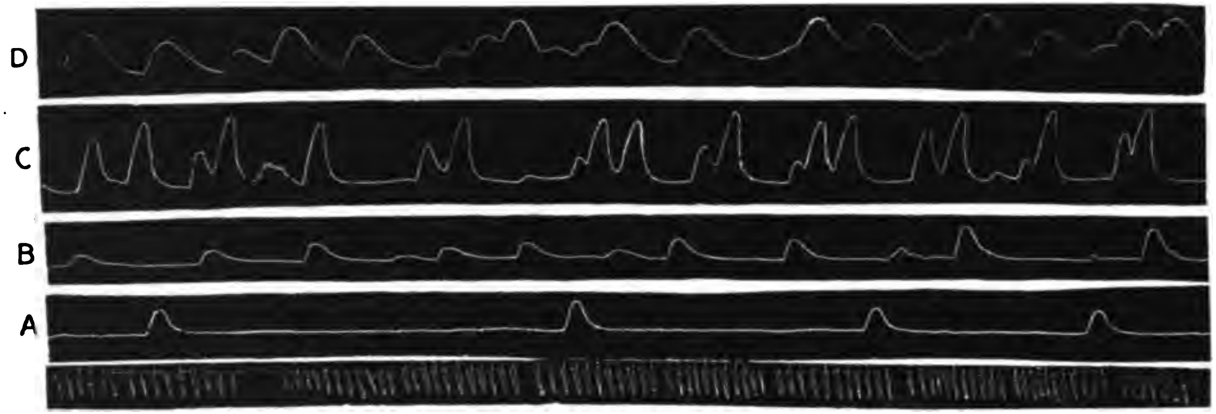

Fig. 4. Water manometer tracings of the rhythmical contraction of the turtle's lung after complete curare, abolishing skeletal and respiratory movements. Tracings *A* to *D* taken successively about 90 minutes apart. Time, 5 seconds. Showing the usual increased rate and amplitude of the lung contractions with increasing asphyxia of the brain.

The fact that the lung rhythm appears in completely curarized animals shows that it is not due to reflexes evoked by acts of external respiration. It shows further that central and efferent nervous processes of the external respiration go on during the curarized state, because these lung contractions are side events in the normal respiratory act.

These lung contractions following the external respiratory act have been studied and described by Francois-Franck (3), (4) in several species of reptilia. They are evidently identical with the active lung contractions following the respiratory act in frogs and salamanders, recently reported by us (13) and (14). It may be of some interest to note that in this regard the lung physiology of amphibia and reptilia is similar or identical, despite the fact that the mechanisms for effecting lung ventilation in these two groups of animals are entirely different.

2. As already noted, the lung contractions develop during the respiratory pause, and if the respirations come close together they may not appear at all, except in states of marked asphyxia. In fact, a lung contraction once begun may be weakened or cut short by an act of external respiration coming on before completion of the contraction. It would thus appear that while a single respiratory discharge from the respiratory center will, as a side effect, induce a lung contraction after a characteristic latent period, several respiratory discharges following in succession closer than this latent period of the lung contraction interferes with this development in such a way that the lung contraction follows the last respiratory act only. We have made very great efforts to determine the mechanism of this interference by satisfactory experiments.

On most of our tracings from the isolated lung, the animal breathing spontaneously by the lung on the intact side, there appears a tonus relaxation of the isolated lung during the period of external respiration, that is, prior to the active lung contraction. This may be seen in figure 3, *A*, and figure 6, *A*. If all purely mechanical factors were excluded, this would indicate a central inhibition of the lung tonus by discharges from the respiratory center prior to the central processes initiating the lung contractions. Francois-Franck (4), (5) has called attention to the fact that on stimulation of the peripheral end of the incompletely isolated vagus the lung contraction produced by this stimulation may be preceded by an apparent inhibition of the lung tonus, in reality due to mechanical factors developed in the body cavity as the result of the contractions of some muscles of the neck. Furthermore, if the isolated and inflated lung presses against any structure in the body cavity with a force equal to or greater than the internal pressure maintained in the lung (1½ to 2 cm. water) and if these structures are so moved by the act of spontaneous inspiration that the pressure on the outside of the lung is decreased, the net result would be an apparent inhibition of the tonus of the isolated lung during the external respiratory acts. We endeavored to eliminate this source of error by complete curarization, by section of the spinal cord below the medulla, and by suspending the isolated lung partly outside the body cavity. In completely curarized preparations there is, of course, no external respiratory act, but the facts cited above go to show that the discharge from the respiratory center, after an initial paralysis, is resumed and continues rhythmically. Despite this fact inhibition of the lung tonus prior to the lung contractions rarely if ever appears on the tracings from our completely curarized animals. This may be due, however, to a depression of the medullary centers by the drug (direct chemical action, or indirect by altering or diminishing afferent skeletal nervous impulses) to such an extent that the tonus motor action of the medullary centers on the lung is abolished. Without such tonic motor action there could be no relaxation of lung tonus by central inhibition. This negative result on completely curarized animals is therefore no criterion of what the discharges from the respiratory center may do in the direction of central inhibition of lung tonus, when such central motor tonus is present.

We have secured tracings from animals partly curarized, from animals with section of the spinal cord in the neck, and from animals without curare or spinal cord transection, but with the observed lung suspended outside the body cavity, *indicating a central inhibition of the lung tonus by the discharge from the respiratory center, an inhibition prior to the motor innervation of the lung.* A tracing illustrating this fact is shown in figure 6, *B*. Water manometers are usually not delicate enough to disclose the inhibition. This inhibition is probably proportional to the amount of central motor tonus in the lungs.

From the point of view of utility or teleology this inhibition would be expected, as greater relaxation of the lung tonus means less resistance to lung inflation, and active lung contraction would counteract the inspiratory effort. The possible utility of the active lung contraction following the respiratory act is another matter. Useless, useful or harmful, it is there, and we are at present concerned only with the mechanisms of its initiation and control.

3. We desire to point out, however, that the above described relations of the external respiratory act to the active lung contractions are similar to the relation of the swallowing act to the peristalsis of the esophagus. If the swallowing acts are sufficiently far apart, each swallowing is followed by a peristaltic wave of the esophagus initiated from the medulla. If the swallowing acts are repeated at very brief intervals esophageal peristalsis follows the last swallowing act only. The parallel between swallowing-esophageal peristalsis and respiration-lung contraction appears complete, despite the very diverse functions of the two mechanisms. The lung has the same organogenesis as the esophagus, and the primitive mechanism for external respiration is swallowing, the active lung contractions following the respiratory act (air swallowing) serving probably a real respiratory function. As the mechanism of external respiration changes with the reptilia the organogenesis and primary nervous relations of the lung remaining the same, *the lung inhibition and contractions associated with the respiratory act may represent inherited mechanisms useless, if not at times actually harmful to animals provided with this later respiratory device. It may be a vestigial mechanism on the road to elimination in the process of evolution.*

4. The influence of asphyxia on the bulbar center for the lung rhythm appears to run parallel with the influence of asphyxia on the respiratory center. In curarized preparations permitted to go into varying degrees of asphyxia the lung rhythm increases in rate and intensity to the point of incomplete lung tetanus parallel with increasing asphyxia (fig. 4). It is clear that asphyxia increases the lung tonus by central motor action. The lung rhythm does not end in this state of incomplete tetanus. If the state of asphyxia is permitted to continue, the incomplete tetanus stage is followed by feebler lung contractions appearing at greater and greater intervals (5 to 10 minute intervals in some cases) before the final quiescence. This probably represents the well-known final feeble activity of the respiratory center when the asphyxia is carried beyond the point of stimulation to that of actual impairment of the center.

Further evidence of this complete parallel is furnished by the type of experiments illustrated in figure 5. Here artificial respiration through the intact lung, normal, *A*, and denervated, *B*, both preparations being completely curarized, inhibits the tonus and rhythm in non-asphyxiated preparations, *A*, evidently by inducing apnea, and diminishes the tonus and incomplete tetanus in the asphyxiated preparation, evidently by decreasing the asphyxial condition of the blood.

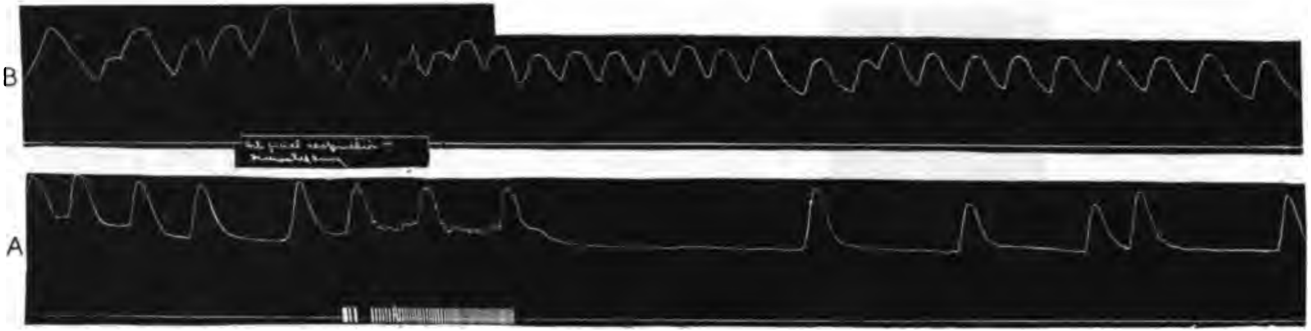

Fig. 5. Water manometer tracings of the intrapulmonic pressure in the turtle. Animals decerebrated and completely curarized; record from left lung isolated except pulmonary vagi branches and lung blood vessels. Cannula in tip of lung. *A:* right lung intact; signal, artificial respiration in right lung, showing inhibition of lung rhythm of the isolated lung. *B:* right lung intact but isolated from central nervous system by section of right vagus in neck. Signal, artificial respiration in right lung. Showing inhibition of the incomplete tetanus of the left isolated lung, through aeration of the blood.

In non-curarized preparations, in which one can follow the action of the respiratory center by means of the external respiratory acts, contractions of the isolated lung, not related to any external respiration, may appear during asphyxia. It appears that asphyxia may occasionally disorganize the normal correlation between the respiratory center and the vagi center controlling the lung contractions, and that asphyxial conditions are capable of stimulating the latter centers directly.

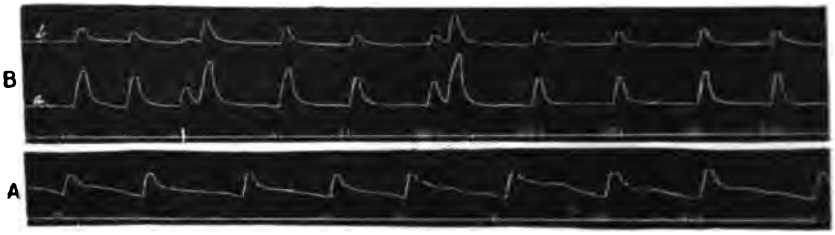

Fig. 6. Tracings of the intrapulmonic pressure in the turtle. Left lung, isolated except pulmonary nerves and blood vessels. Left bronchus ligated. Cannula in tip of lung. Animals decerebrated. Right lung left in normal relations. *A:* signal, spontaneous respiration. Showing inhibition of tonus of isolated lung during respiratory movements followed by strong contraction of lungs. Water manometer. *B:* animal partially curarized, having respiratory muscles in feeble activity. Signal, spontaneous respiratory movements. Both tracings from left isolated lung. *a*, record from delicate tambour; *b*, water manometer. Showing lung contractions following spontaneous respiratory movements, preceded by tonus inhibition, as revealed by tambour, the water manometer not being delicate enough to register the tonus inhibition.

THE RÔLE OF THE EFFERENT PULMONARY VAGI FIBERS AND THE VARIOUS PARTS OF THE BRAIN IN THE GENESIS OF THE RHYTHMIC LUNG CONTRACTIONS

1. In the species of turtle studied by us the spontaneous lung contractions are of central origin, and the motor innervation is through the vagi, as shown by Kahn (8) and François-Franck (5) for the common land turtle of Europe. This may be shown by experiments such as reproduced in figure 7, *A*. In that case the animal was completely curarized, graphic records being taken of the lung contractions both from the isolated and the intact lungs. The intact lung has, of course, its sympathetic nerve connection intact. Nevertheless section of the vagus nerve on the side of the intact lung abolishes the strong contractions in that lung at once and forever. The section of one vagus

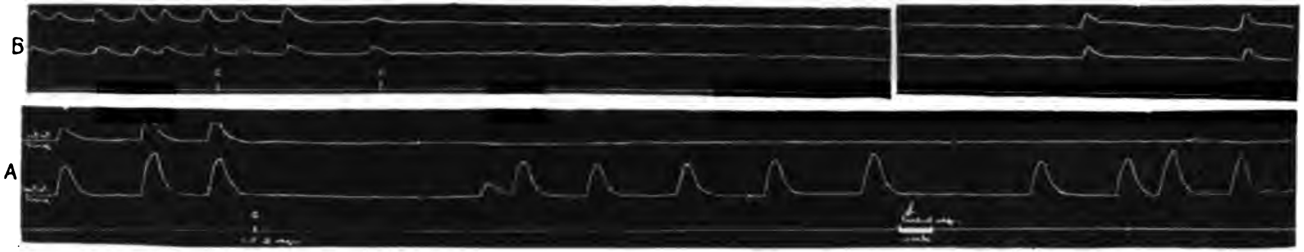

Fig. 7. Water manometer tracings of the intrapulmonic pressure in the turtle. *A* and *B*: upper tracing in each case, right or intact lung; lower, left or isolated lung. Animal completely curarized, after previous decerebration. *A*: *a*, section of right vagus in neck showing complete abolition of rhythmic lung contractions in right lung and temporary inhibition (reflex) of contraction of left lung. *b*, stimulation of central end of right vagus with weak tetanizing current, showing reflex inhibition of the left lung, that is, inhibition of the medullary center. *B*: *a*, beginning isolation of spinal cord in the neck. *b*, section of spinal cord in neck, showing reflex inhibition ("shock") of the medullary center governing the rhythmic lung contractions.

stops the contraction of the lung on the opposite side temporarily through stimulation of afferent inhibitory fibers in the vagus trunk depressing the medullary centers. It is thus clear that the motor innervation of the lung rhythm is solely through vagi nerves. The same fact can be illustrated by the use of atropine. Atropine paralyzes the vagi motor fibers in the lungs. The tracing reproduced in figure 8, *C*, was secured from the left lung of a completely curarized preparation, the lung being isolated from the body except for the pulmonary blood vessels and pulmonary vagus branches. Intravenous injection of 0.5 mgm. atropine sulphate abolished the lung rhythm promptly just as it abolished the motor action of the vagus on the lung musculature.

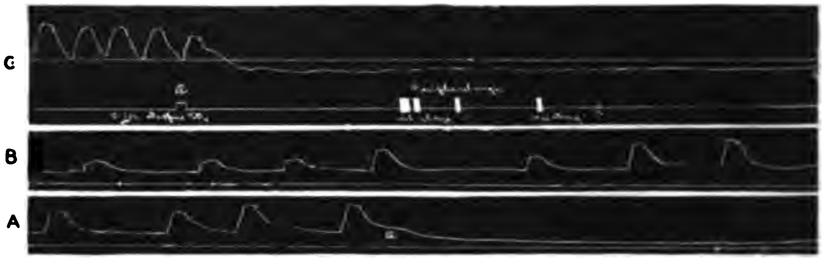

Fig. 8. Water manometer tracings of the intrapulmonic pressure in the turtle. Animals decerebrated. Left lung isolated, cannula in tip; right lung in normal relations. *A* and *B*, showing lung contractions following the spontaneous respiratory movements. *A*: *a*, section of right vagus in neck, followed by temporary inhibition (central) of the tonus and contractions of the opposite lung, also temporary inhibition of the respiratory movements. The latter reappear at *X*, but are not followed by lung contractions. Tracing *B* is continuation of *A* after a 6 minute interval, the lung contractions gradually regaining normal vigor. *C*: animal completely curarized; lung in strong tonus and contractions, probably from partial asphyxia. *a*, intravenous injection of ½ mgm. atropine sulphate followed by permanent inhibition (peripheral action) of lung tonus and contractions. Signal shows vagus stimulation is without effect on lung.

These tracings indicate a tonic condition of the medullary center controlling the lung motor tissues. This center is more profoundly influenced by depressant impulses ("shock"?) than is the respiratory center.

The reader's attention may again be directed to the fall in lung tonus induced by peripheral vagus paralysis of atropine and by afferent depressor impulses acting on the medulla (fig. 8, *A* and *C*). The tracings in figure 8 show at least that under some conditions (partial asphyxia) the medulla-vagi-lung motor mechanism is in a state of tonic activity, in which the stronger rhythmic contractions are superimposed much like the constant tonus and the occasional peristaltic contractions of the gut. We have so far been unable to demonstrate this tonic activity under more nearly normal conditions; but our results point in the direction of some normal motor tonus.

2. The inhibition of the contractions of the lung on the opposite side induced by section of one vagus (afferent inhibition of a central automatism) is usually more prolonged than shown in figure 7, *A*. In preparations not curarized it can be shown that section of one vagus temporarily inhibits both external respiration and the tonus and contractions of the lung on the opposite side, the external respiratory acts returning sooner than the lung contractions.

Section of the spinal cord in the neck also produces a very prolonged inhibition of the medullary center initiating the lung rhythm (fig. 7, *B*). This central inhibition or shock is shown not only by the temporary abolition of the spontaneous rhythm, but also by the lowering of the excitability of these centers to reflex stimulation leading to lung contractions.

3. As reported by many previous investigators, stimulation of the peripheral end of the vagus nerve in the turtle causes contraction of the lung musculature (fig. 9, *C*). We have never seen any inhibitory effects on the lung in the turtle from stimulation of the peripheral vagus. This appears to us significant in view of the fact that in the frog the predominant action of the vagi on the lungs is inhibitory, and in the most primitive amphibian lung (necturus) all the efferent lung nerve fibers appear to be inhibitory.

In the species of turtle studied by us the motor action of the vagi on the lungs is strictly unilateral. Single induction shocks (make or break) applied to the peripheral vagus will not cause lung contractions unless exceedingly strong. Weak tetanizing currents applied to the vagi induce an incomplete tetanus or rhythm that seems to indicate a certain degree of refractory state in the peripheral mechanism. Strong tetanization of the vagi will, however, induce curves of complete tetanus closely resembling those of an ordinary muscle nerve preparation (fig. 9, *C*).

In view of the identity of origin and certain similarities in the nervous control of the lung and the alimentary canal, we made some comparisons between the vagi motor action on the lungs and on the stomach (fig. 10). The latent period of the vagi motor action on the stomach is very much longer than its motor action on the lung. The turtle's stomach cannot be tetanized by vagus stimulation; the lungs can be tetanized. The third marked difference is the quick failure of vagus motor action on the stomach on repeated stimulation in comparison with the repeated tetany of the lungs that may be induced by vagus stimulation. These differences may be due, in part, to the fact that in the turtle the vagi carry inhibitory efferents to the stomach in addition to the motor, while the efferent vagus lung action is solely motor. The differences also suggest that the gut has retained more of its primitive automatism (independent peripheral nervous system) while the differentiation in the lung has been toward the more simple relations of a muscle nerve preparation.

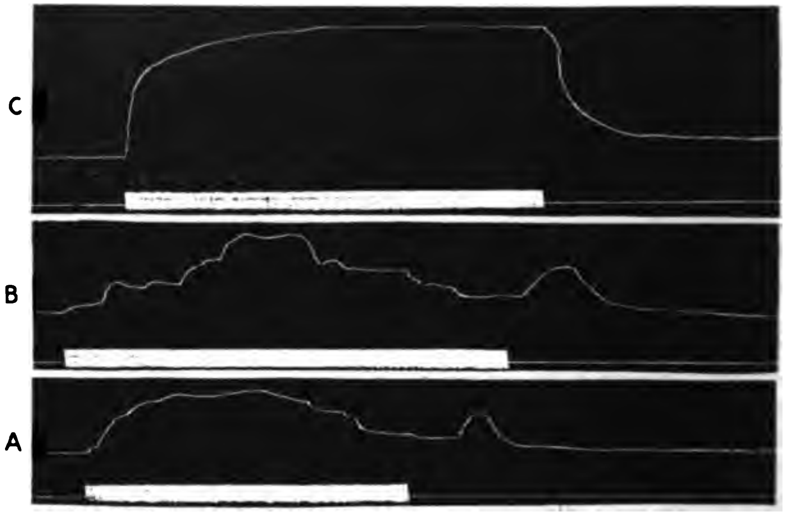

Fig. 9. Water manometer tracings of the contractions of the isolated lung of the turtle. Animal decerebrated and completely curarized; left lung isolated except for pulmonary vagi fibers and blood vessels. Cannula in left bronchus. Stimulation with moderately strong tetanizing current, *A*, optic lobes, *B*, medulla, *C*, peripheral end of left vagus, showing incomplete and complete tetanus of the lung musculature.

4. In 1878 Martin reported that stimulation of the optic lobes in the frog accelerates the external respiratory movements. Since that time several investigators have concerned themselves with the problem of accessory respiratory centers above the level of the medulla. Recently Coombs (12) reported that stimulation of the optic lobes or the medulla in the turtle with weak tetanizing current causes lung contractions via the vagi nerves.

As we have shown, the normal rhythmic contractions of the turtle lung are side events in the external respiratory act. This being the case, we would expect lung contractions from stimulation of any part

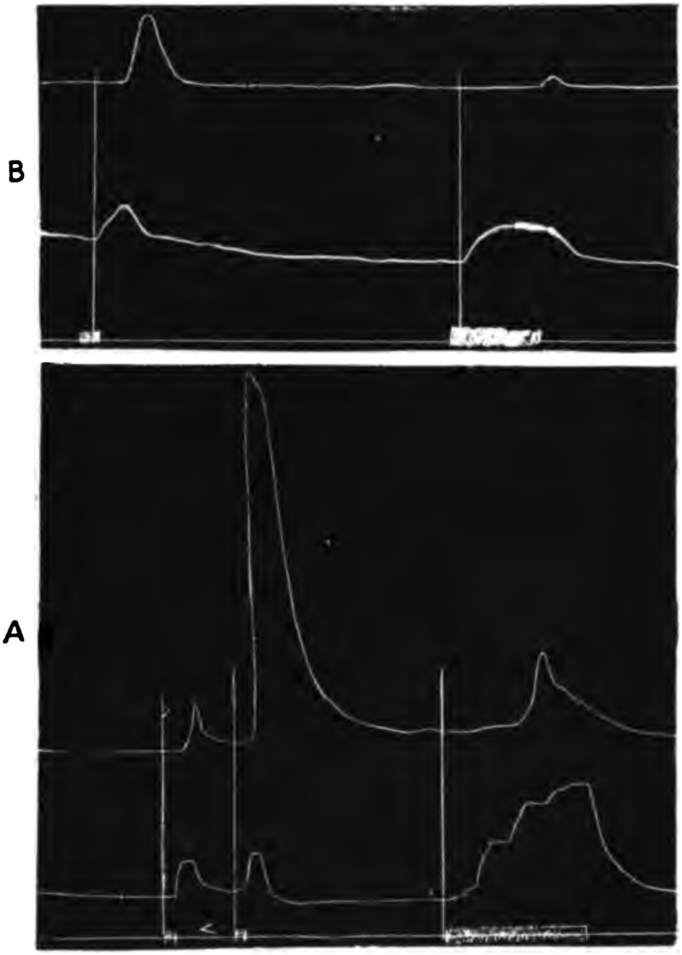

Fig. 10. Simultaneous water manometer tracings of the stomach and the lung contractions on stimulation of the peripheral end of the vagi nerves in the turtle. In *A* and *B*, upper record from stomach (balloon method); lower record from left lung, isolated except for the pulmonary blood vessels and vagi fibers; cannula in left bronchus. Signal, stimulation of peripheral end of left vagus with the tetanising current. Showing longer latent period, more rapid fatigue and absence of tetanus in the gastric response to vagus stimulation.

of the central nervous system that has motor connections with the medullary respiratory center. This has turned out to be a fact, at least as regards the optic lobes. In figure 9, *A* and *B*, are reproduced typical tracings of the incomplete tetanus the lung induced by stimulation of the optic lobes and the medulla with weak tetanizing currents. The lung contraction curves induced from the optic lobes and from the medulla in completely curarized turtles are practically indistinguishable. Both centers fail quickly under this type of stimulation and there is a single after-discharge (lung contraction) that appears more normal than the contractions induced directly by the stimulation. Complete tetanus of the lungs cannot be induced by central stimulation, evidently because of central refractory states.

A tonus rhythm or spontaneous contraction of the lung after section of the vagi nerves similar to that described by Fano and Fasola (2) in the European turtle, has been seen occasionally in our turtle preparation, when we used an exceedingly delicate tambour as a recording device. The contractions are feeble but slow and regular. They may persist for hours (fig. 23, *A*). It is probable that a peripheral automatic motor mechanism of the lung is present in all species of turtle, although it may differ in degrees in various species; but it requires not only delicate recording devices but, above all, animals in prime condition to reveal it. It is certain that parallel with the development of the new type of external respiratory mechanism in the reptilia, the peripheral lung motor automatism, so prominent in the amphibia, has retrograded or has been changed to one of primarily medullary origin.

LUNG REFLEXES

1. Lung reflexes from sensory stimulation of the respiratory tract: a. The pulmonary branches of the vagi contain two kinds of afferent fibers acting on the medullary center, one type causing reflex lung contraction in a quiescent preparation, or acceleration of the contractions in an active preparation. The other type causing inhibition of the tonus in the case of quiescent preparations or of the contractions in active preparations. For the sake of brevity we call these motor and inhibitory afferents, respectively. The motor afferents are stimulated by inflation as well as by deflation of the lung (fig. 11) or by the weak tetanizing current. Strong tetanizing currents, on the other hand, stimulate the inhibitory afferents. In all these experiments the lung inflation and deflation, and the direct stimulation of the central and of the pulmonary

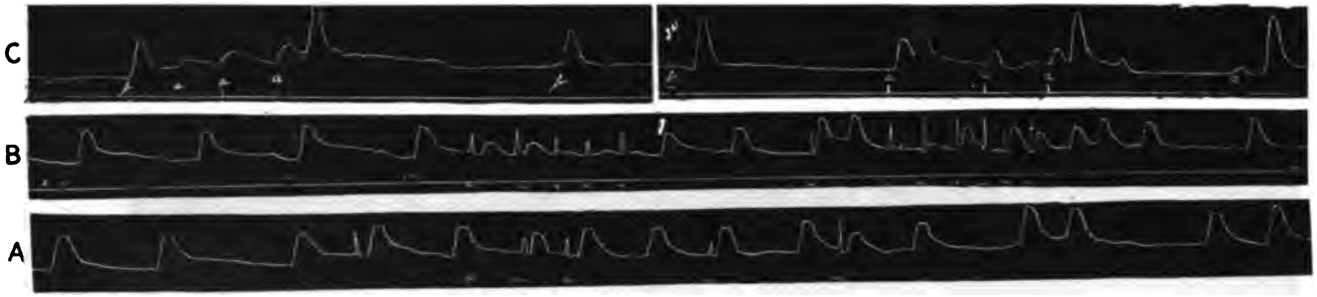

Fig. 11. Water manometer tracings of the intrapulmonic pressure in the turtle. All records from the left isolated lung; cannula in tip of lung. Animals decerebrated. Right lung intact. *A:* Animal completely curarized and showing contractions of isolated lung. *a*, single inflations (positive air pressure) in the intact lung, showing reflex contractions of opposite lung. *B:* animal not curarized. Signal, spontaneous respiration; *a*, single inflation of right or intact lung, showing reflex contractions and also evidence of central refractory state. *C:* Animal completely curarized, spontaneous lung rhythm absent; *b*, single inflation (positive pressure); *a*, single deflation of right or intact lung, showing reflex contraction into opposite lung both from deflation and inflation.

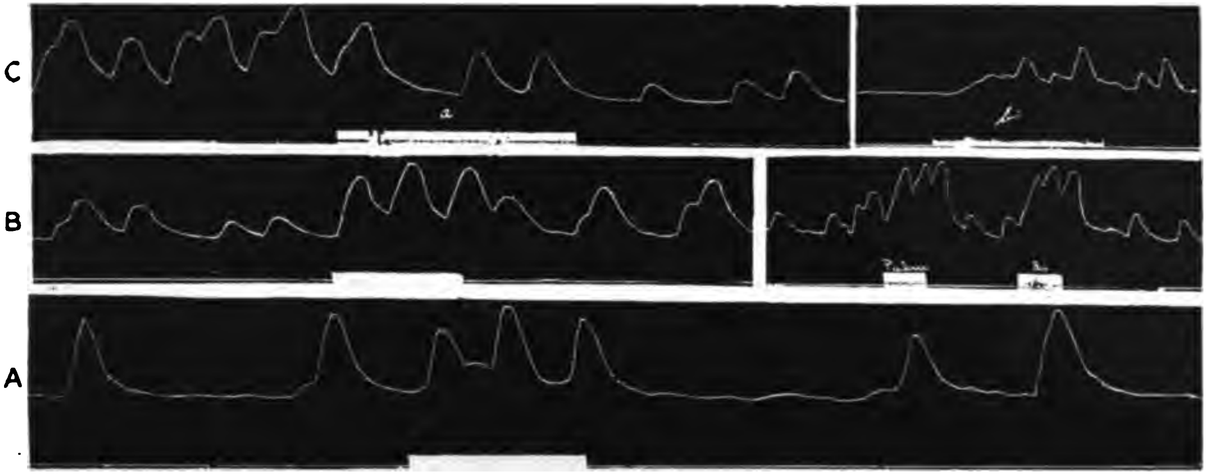

Fig. 12. Water manometer tracings of the intrapulmonic pressure in the turtle. Animals decerebrated and completely curarised. Records from left lung, isolated, except for pulmonary blood vessels and nerves. Cannula in tip of lung; bronchus ligated. *A* and *B:* stimulation of central end of pulmonary branches of right vagus with weak tetanising current, showing reflex augmentation of the spontaneous lung rhythm. *C:* stimulation of central end of pulmonary vagi (right) with strong tetanising current, showing inhibition of the spontaneous lung rhythm, *a*, and initiation of this rhythm in the quiescent preparations *b*.

vagi, we used, of course the lung and vagus on the opposite side to the lung serving to record the contractions. In no case did we note the inhibitory afferents being stimulated by lung inflation or lung deflation.

Typical tracings showing the reflex lung contraction on lung inflation and deflation are given in figure 11. Tracings showing the opposite reflex effects on weak and on strong tetanization of the central end of the pulmonary vagi are reproduced in figure 12. The pulmonary motor afferents can also be stimulated by other mechanical means, for example, rubbing the collapsed lung of one side between one's fingers induces reflex contraction in the opposite side via the medulla.

Reflex lung tetanus cannot be produced by lung deflation or inflation, that is, the active change in the intrapulmonic pressure, and not the state of continued inflation or collapse that acts as stimulus to the motor afferents.

In preparations not curarized, but breathing normally by means of the intact lung, single inflation of the intact lung during a respiratory pause usually gives a reflex lung contraction; but if the inflation is made shortly after completion of a spontaneous contraction by the isolated lung, the reflex may not be elicited. The same is true if a series of lung inflations is produced with very short intervals between each inflation. These facts evidently point to a condition of refractory state of the medullary reflex center, unless the phenomenon can be due to simultaneous stimulation of the inhibitory pulmonary afferents.

In turtle preparations in good condition deflation of one lung by suction through the trachea invariably initiates or accelerates the external respiratory movements. Lung inflation, on the other hand, may start a respiratory movement or it may temporarily inhibit the external respiration. It is thus evident that stimulation of the pulmonary motor afferents by lung inflation may initiate reflex lung contractions by acting on the medullary motor center directly, the discharge of the respiratory center not being a necessary link in the chain.

We have found that a single inflation of the intact or isolated lung aerates the blood, accelerates the heart and by so doing improves the circulation through the medullary centers because of the increase in the general arterial pressure. It is certain however that the contraction of the isolated lung following one or more inflations of the intact lung does not occur because of the improved circulation of a more highly oxygenated blood through the medullary lung center; for this reflex contraction persists not only following the inflation of a lung the pulmonary vessels of which have been occluded by a temporary ligature but can be elicited for a considerable time after the complete excision of the heart. On the other hand, it is not obtained from the isolated lung following a single or several inflations of the denervated but otherwise intact lung of the opposite side.

We designate the reflex inhibition of lung tonus and contraction by strong tetanization as due to a separate type of sensory fibers, the inhibitory afferents, at the same time keeping in mind the possibility that this inhibition may in reality be due to abnormally strong or abnormally rapid series of impulses over the motor afferents impinging on the central automatic mechanism. This stimulation also inhibits the external respiratory movements.

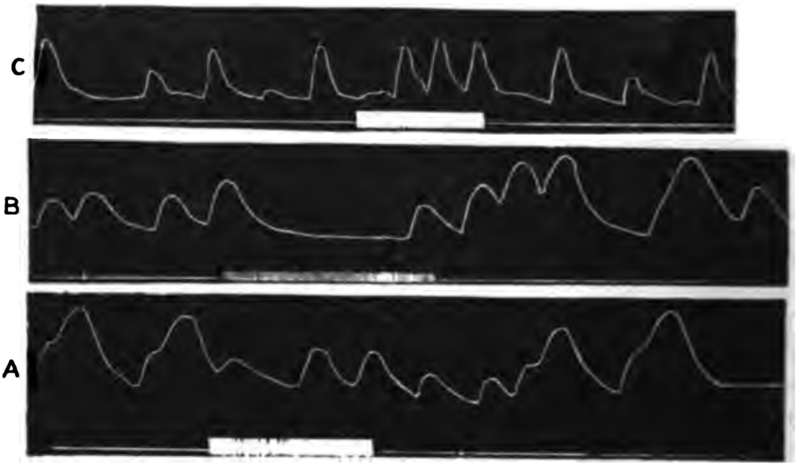

Fig. 13. Water manometer tracings of the intrapulmonic pressure in the turtle. Animals decerebrated and completely curarized. Records from left lung isolated except from pulmonary blood vessels and vagi branches. Cannula in left bronchus. *A* and *B:* stimulation of central end of recurrent laryngeal nerve, showing reflex inhibition of the lung rhythm. *C:* stimulation of central end of gastric branches of right vagus, showing reflex augmentation of the lung rhythm.

b. Stimulation of the central end of the inferior or recurrent laryngeal nerves causes invariably reflex inhibition of the lung tonus and contractions (fig. 13, *A* and *B*). At no time was reflex lung contraction obtained from the central end of this nerve. Motor afferents for the lung reflex are either absent or the inhibitory afferents are so predominant that the action of the former type is entirely suppressed on simultaneous stimulation of the two types.

Observations on the effects of external respiration of stimulation of the central end of the inferior laryngeal were not made but in connection with this reflex depression of the lung contraction mechanism we can state that mechanical tension or pulling on the trachea causes profound depression of the respiratory center (fig. 14). Mechanical stimulation of the larynx and glottis (rubbing with blunt probe) on the other hand induces strong lung contractions.

c. Mechanical stimulation (gentle rubbing) of the posterior nares causes very powerful lung contractions. If the stimulation is long-continued the lung goes into incomplete tetanus. This stimulation initiates lung contractions in quiescent preparations and accelerates the rhythm in active preparations. When the nares are thus stimulated in decerebrated but non-curarized preparations such stimulation induces most violent respiratory efforts and general struggling.

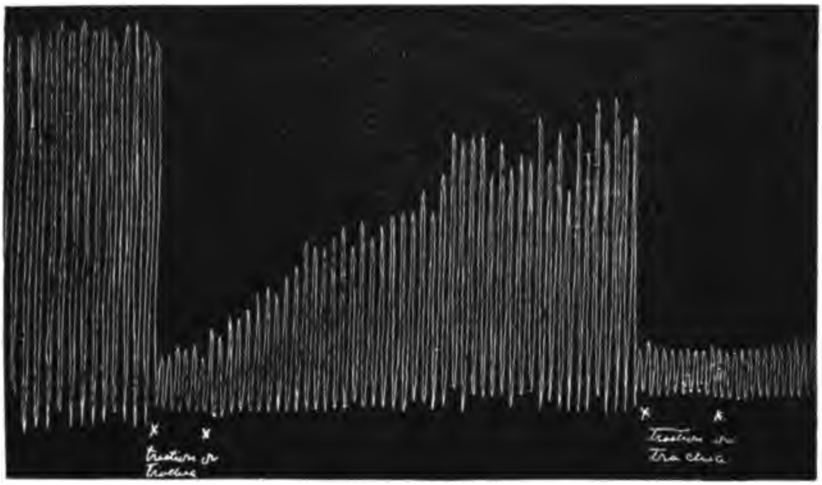

Fig. 14. Tracing of respiratory movements (water manometer) in the turtle. Animal decerebrated; cannula in trachea, rapid respiration due to partial asphyxia; x–x', strong forward traction on trachea, inducing a temporary depression of the respiratory center, probably through stimulation of the recurrent laryngeal nerves.

In general the reflex lung contractions evoked from the nares are more powerful than those produced by stimulation of any other region of the respiratory tract. It is one of the last lung reflexes to disappear as the preparation deteriorates from circulatory failure, and other causes, in long continued experiments. Reflex lung contractions from the nares can be obtained in preparations with the medullary center in such poor condition ("reflex shock") that the spontaneous external respiratory act is not accompanied by lung contraction, all of which point to the fact that the sensory nerve fibers of the posterior nares make strong motor connection with the medullary center controlling the lung contraction. Stimulation of the posterior nares with chemical irritants were not tried.

2. Lung reflexes from spinal sensory nerves: Weak tetanizing of the central end of any spinal nerve causes reflex lung contraction. Most of the tests were made with the sciatic and the large brachial nerves. Weak stimulation accelerates the rhythm in an active preparation and initiates a rhythm in quiescent preparations (fig. 16, *A*, fig. 17, *A*). Strong stimulation induces incomplete lung tetanus (fig. 16, *B* at *x-x'*, fig. 17, *C*). In quiescent preparations the incomplete lung tetanus may be followed by brief periods of apparently spontaneous lung rhythm (fig. 17, *B*).

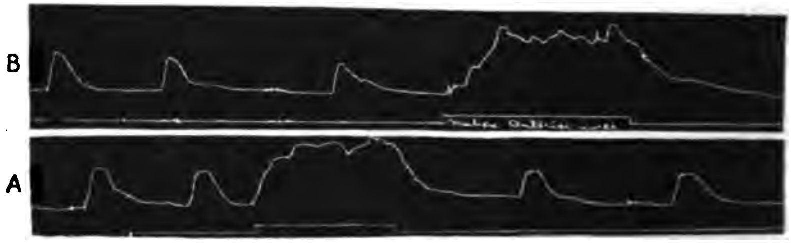

Fig. 15. Water manometer tracings of the intrapulmonic pressure in the turtle. Records from isolated left lung, cannula in left bronchus, pulmonary vagi fibers and blood vessels being intact. Animal decerebrated. *A*, animal completely curarized; *B*, animal not curarized. Signal, mechanical stimulation of the nares, showing incomplete tetanus (reflex), of greater amplitude than that of the rhythmical lung contractions.

If the preparations are in good condition gentle rubbing of the skin causes lung contractions. Pinching, crushing or cutting the skin causes powerful motor reflexes into the lung (fig. 16, *C*), even when the reflex excitability is not at its maximum. Strong lung contractions are likewise induced by mechanical (rubbing with a blunt probe) or electrical stimulation of the cornea.

Reflex inhibition of the lung tonus or the rhythmical contractions were not obtained from the spinal sensory nerves by any mode of stimulation.

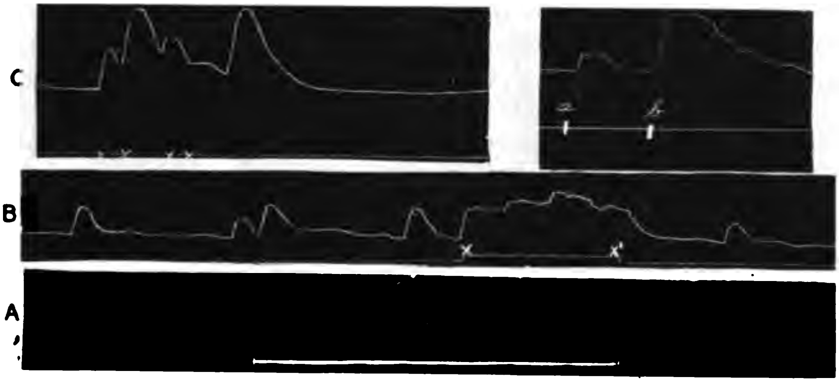

Fig. 16. Water manometer tracings of the intrapulmonic pressure in the turtle. Animals decerebrated and completely curarized. Records from left lung, isolated except for pulmonary blood vessels and vagi nerves. Cannula in tip of lung, bronchus ligated; *A:* light stimulation; *B:* stronger stimulation of central end of sciatic nerve, showing acceleration and incomplete tetanus of lung rhythm. *C:* *a*, stroking of skin of hind leg with finger; *b*, pinching toes of hind leg, *x*, cutting skin of hind leg. Showing reflex lung contraction on stimulation of cutaneous nerves.

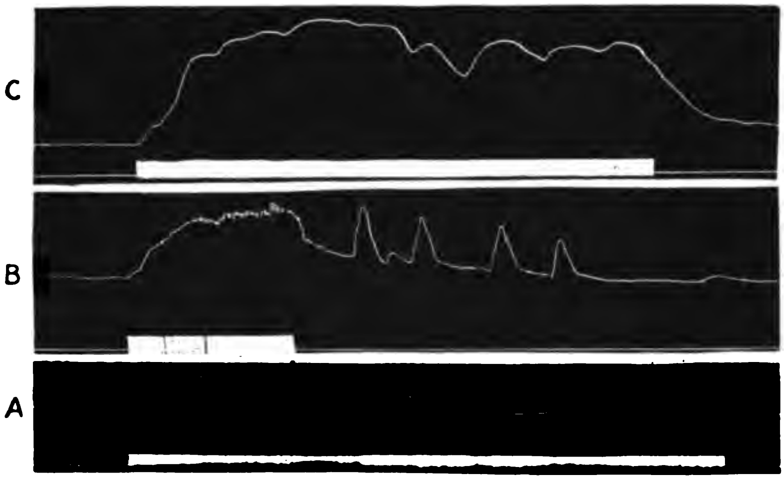

Fig. 17. Water manometer tracings of the intrapulmonic pressure in the turtle. Animals decerebrated and completely curarized. Records from left lung, isolated, except for pulmonary blood vessels and nerves. Left bronchus ligated; cannula in tip of lung. Preparations showing no spontaneous medulla-lung rhythm. Signal, stimulation of central end of sciatic nerve with tetanizing current. Showing reflex development of lung rhythm and lung tetanus.

3. Lung reflexes from the visceral sensory nerves: a. The alimentary tract. Reflex lung contractions may be obtained from practically every part of the alimentary canal, the most powerful being the lung contractions following stimulation of the lower end of the gut (cloaca, rectum, large intestine). Mechanical distention with balloon, rubbing, pressure, cutting or crushing this end of the alimentary canal causes contractions in the lung. (fig. 18, *B*, *C*, *Da*, *Dd*; fig. 19, *a*, *c*). In fact, one may induce incomplete tetanus of the lungs by strong stimulation of the lower end of the gut.

Mechanical or electrical stimulation of the small intestines also induces lung contractions (fig. 19, *b*, *d*). Similar lung reflexes are induced by stimulation of the central end of the gastric vagi branches (fig. 13, *C*), and by direct stimulation of the esophagus. But the contractions of the empty stomach, even when most vigorous, do not influence the lung motor mechanism.

Inhibition of the lung tonus and contractions were not seen as a result of artificial stimulation of the gut. But it seems clear that the afferent nerves of the alimentary canal, especially the oral and anal ends, make motor connections with the lung medullary centers.

b. The genito-urinary tract. Powerful lung contractions are induced by mechanical distention, pinching, crushing, tearing or electrical stimulation of the urinary bladder (fig. 18, *A*; fig. 19, *c*, *b*). Electrical or mechanical stimulation of the ureters, penis, prepuce and testis also produces motor reflexes into the lungs (fig. 18, *Dc*; fig. 19, *f*, *j*). As in the case of the alimentary tract, no reflex inhibitions into the lung were secured from the genito-urinary tract. In several female specimens the ovaries and the oviducts were stimulated, without any reflex influence on the lungs. Stimulation of the kidneys also yielded nothing definite in the way of lung reflexes. The most powerful and consistent lung reflexes in this group are those elicited from the urinary bladder.

c. The visceral sympathetic nerves. Outside the alimentary, the genito-urinary and the respiratory tracts no systematic or long-continued work was made on possible lung reflexes from the viscera, except for the stimulation of the central ends of the main visceral sympathetic nerves and connections. We may note, however, that the stimulation of the central end of the cardiac vagi branches has no effects on the lung. Reflex lung contractions were, on the other hand, obtained from the gall bladder, liver (fig. 19, *g*), and from the spleen.

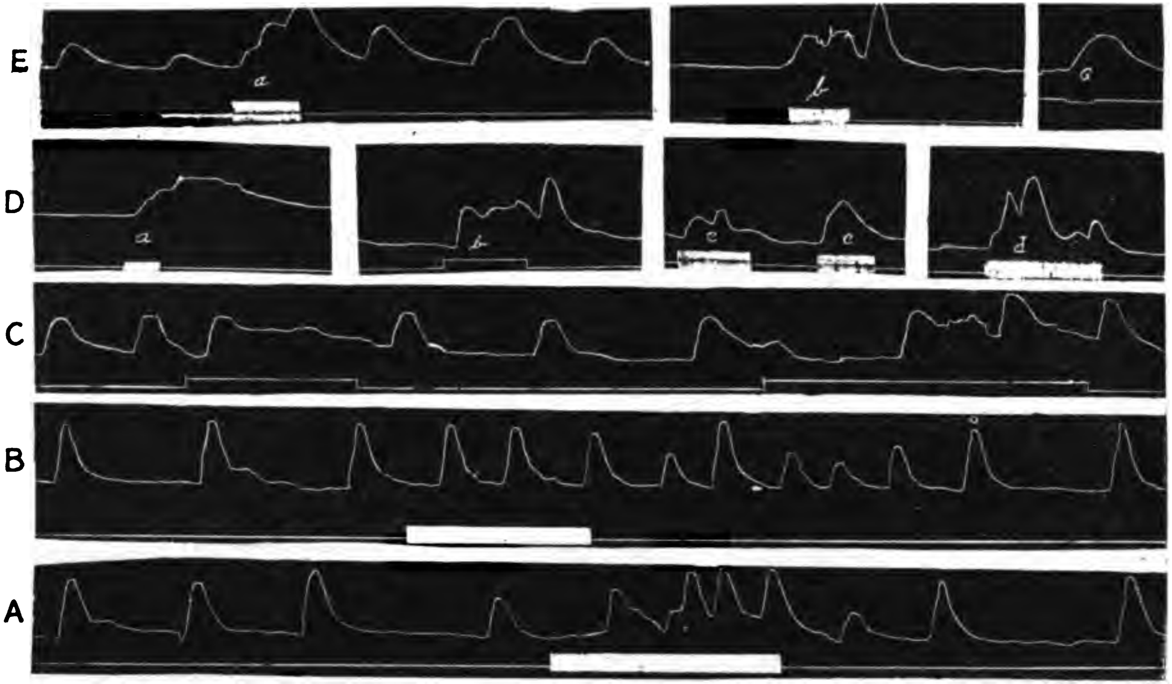

Fig. 18. Water manometer tracings of the intrapulmonic pressure in the turtle. Animals decerebrated and completely curarized. Records from left lung, isolated except for pulmonary blood vessels and vagi branches. Cannula in left bronchus. *A:* signal, distention of urinary bladder with balloon. *B:* signal, distention of rectum and large intestine by balloon, showing reflex augmentation of the lung rhythm. *C:* signal, mechanical stimulation of cloaca, showing reflex increase of lung rhythm. *D:* preparations showing no spontaneous lung rhythm; *a*, mechanical stimulation of cloaca; *b*, mechanical stimulation (pinching) of urinary bladder; *c*, mechanical stimulation (pinching) of penis; *d*, mechanical stimulation (pressing between fingers) of large intestine, showing reflex lung contractions. *E: a* and *b*, mechanical; *c*, electrical, stimulation of cornea, showing reflex lung contractions.

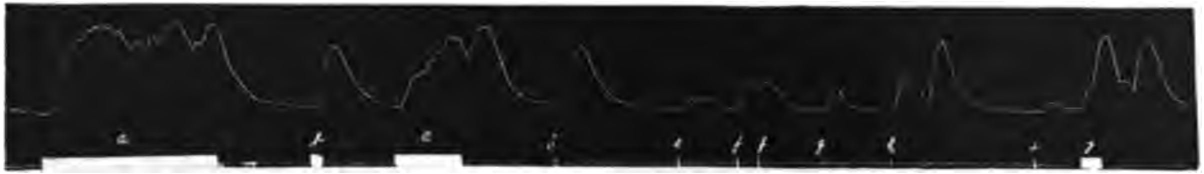

Fig. 19. Water manometer tracing of the intrapulmonic pressure in the turtle. Animal decerebrated and completely curarized. Tracing from left lung, isolated except for the pulmonary branches of the vagus and the blood vessels. Cannula in left bronchus. Preparation showing no spontaneous lung rhythm. *a*, Stimulation of rectum with strong tetanizing current; *b*, stimulation of small intestine, with strong tetanising current; *c*, stimulation of urinary bladder, with strong tetanizing current; *d*, rubbing small intestine between fingers; *e*, cutting large intestine; *f*, pressing testes between fingers; *g*, rubbing urinary bladder between fingers; *h*, tearing wall of urinary bladder; *i*, rubbing tip of right lung between fingers; *j*, strong electrical stimulation of the right ureter. Showing reflex lung contractions and lung tetanus from stimulation of visceral sensory nerves.

The stimulation (electrical) of the central end of the visceral sympathetic nerves was made in a great many preparations, some in good, some in poor reflex conditions. The results are positive, that is, the stimulation of the visceral sympathetic nerves gives reflex lung contractions (fig. 20). The reflex lung contractions are particularly powerful from nerves *2*, *3* and *4* (fig. 2), these probably representing the group of splanchnic sympathetic nerves of the higher animals.

Reflex inhibitions of lung tonus and of lung contractions were never seen as results of stimulation of the central end of the visceral sympathetics.

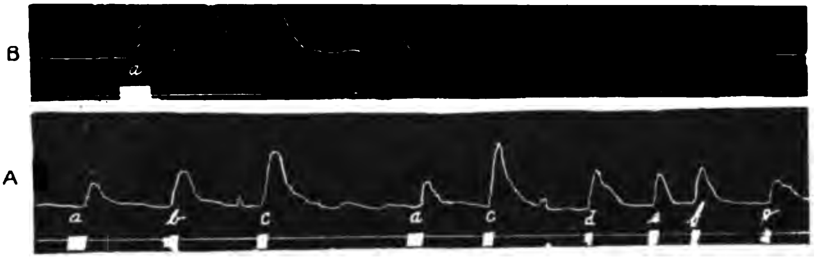

Fig. 20. Water manometer tracings of the intrapulmonic pressure in the turtle, showing reflex lung contractions on stimulation of the central end of various sympathetic nerves. Animals decerebrated and completely curarized. Record from isolated left lung; cannula in left bronchus. Preparations showing no spontaneous lung rhythm. *A:* stimulation with tetanizing current; *a*, nerve 2 (see fig. 2); *b*, nerve 3; *c*, nerves 2 and 3; *d*, nerve 4; *e*, nerve 5; *f*, nerve 6; *g*, nerve 7. *B:* *a*, stimulation with tetanizing current of nerves 2 and 3, showing reflex initiation of a temporary lung rhythm.

Results at times positive at times negative were obtained from the central end of the cervical sympathetic (fig. 2). In some preparations the stimulation of this nerve induced what appeared to be a reflex lung contraction, in other preparations, in equally good reflex condition, the stimulation had no effect on the lung. It appears, therefore, that afferent components in the cervical sympathetic nerve trunk are few in number and variable in their central action, at least as regards their influence on the medullary centers controlling lung tonus and lung contractions.

As the preparations are deteriorating the lung reflexes evoked from the visceral sympathetic nerves usually fail sooner than those called forth by stimulation of the spinal sensory or the afferent nerves of the respiratory tract itself. The cloaca, rectum and urinary bladder are an exception to this rule, the lung reflexes evoked from these organs usually persisting to the end of all reflex response of the animal.

4. The influence of visceral sensory nerves on the respiratory center. We have shown that, excepting the inferior laryngeal (inhibition), the cardiac vagi (no action), the cervical sympathetic (inconstant), and strong stimulation of the pulmonary afferents (inhibition), sensory stimulation, spinal and visceral, induces reflex lung contractions. Since lung contraction is a normal adjunct to the external respiratory act it is pertinent to ask whether these lung reflexes are not secondary to the initiation or acceleration of discharges from the respiratory center. What effects have these sensory stimuli on the rate and intensity of the external respiratory movements? The turtle appears to be no exception to the rule that stimulation of spinal sensory nerves accelerates or initiates external respiration. As regards the spinal sensory nerves, therefore, we have a complete parallel between effects on external respiration and on lung contractions. Both are positive.

In regard to the visceral sensory stimulation the results are not so clear. In the first place much of our reflex work was done on completely curarized animals, preventing parallel observation on the external respiration. We can state, however, that so far as our observations go, stimulation of the abdominal sympathetic nerves induces reflex contraction of the respiratory muscles as an invariable result. But discrepancies appear when we come to the stimulation of some of the visceral organs, especially the cloaca, rectum and urinary bladder. As already pointed out the mechanical or electrical stimulation of these organs causes reflex lung contractions and never any inhibition, at least in curarized preparations. But the same type of stimulation of these organs in non-curarized preparations may cause pure inhibition of the external respiration, as witness the tracing reproduced in figure 21. We are dealing here with two possibilities, viz., *a*, stimulation of some of the visceral sensory nerves induces opposite effects on the two medullary centers depressing the respiratory center and stimulating the lung motor center; or *b*, the visceral afferents may have both effects on the two centers, the end result (depression or stimulation) depending on the intensity and rate of the artificial stimulation, the central action of curare tending to reveal only the stimulation action on the lung motor mechanism. Further work is required to establish either or both of these possibilities.

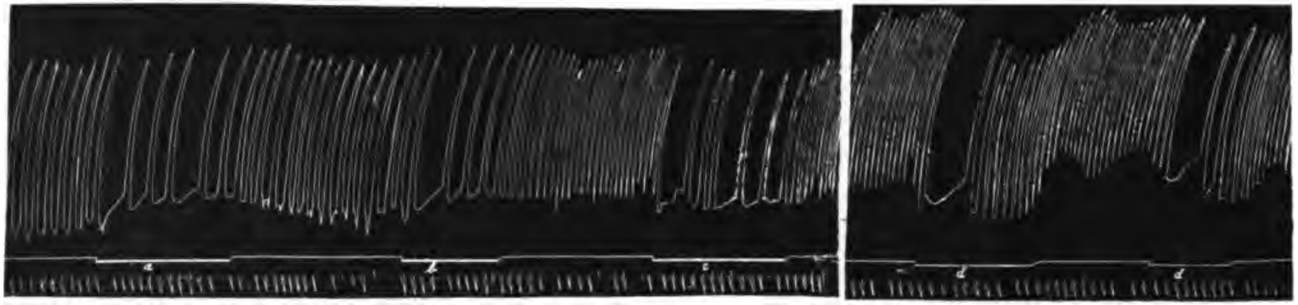

Fig. 21. Water manometer record of respiratory movements in the turtle; animal decerebrated; dorsal shell removed on left side; left lung isolated and left bronchus ligated. Right lung intact; cannula in trachea. *a*, Distention of rectum and lower part of large intestine by inflation of small balloon; *b*, electrical stimulation of the rectum; *c*, distention of urinary bladder by inflation of small balloon; *d*, electrical stimulation of urinary bladder, showing reflex inhibition of respiratory movements by stimulation of afferent nerves in large intestine, rectum and urinary bladder.

RESULTS ON THE SNAKE

1. *Spontaneous lung contractions.* If the external respiratory acts are not too close together, each act of external respiration is followed by a contraction of the lung. These lung contractions are feeble and of very short duration (fig. 22, *A*). If the attempts at external respiration come close together lung contractions appear only occasionally or rather during the respiratory pauses (fig. 27, *Ca*).

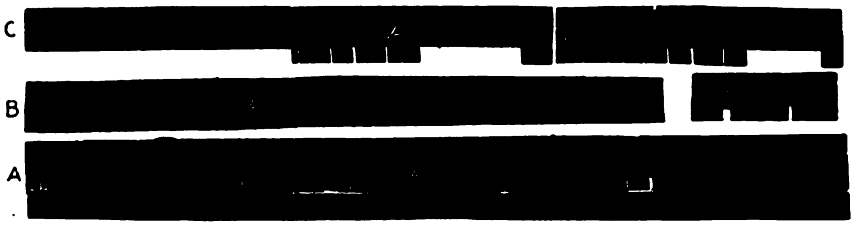

Fig. 22. Tracings (delicate air tambour) of the intrapulmonic pressure in the snake. Spinal cord cut and pithed below the medulla. Lung isolated; cannula in tip, trachea ligated. *A:* signal, spontaneous respiratory movements, showing lung contractions following each attempt at respiration; *a*, artificial respiration through cannula in tip of lung; *b*, pithing of brain followed by cessation of lung contractions. *B: a*, mechanical stimulation of skin of the head; *b*, mechanical stimulation of the cornea; *c*, mechanical stimulation of nares; *d*, electrical stimulation of central end of left vagus—showing reflex lung contractions. *C: a*, lung contractions following spontaneous respiratory efforts; *b*, tetanizing of both peripheral vagi; *c*, stimulation of the peripheral vagi with increasing number of electrical shocks—showing that motor discharge to lungs following respiratory movements is nearly maximal, as brief tetanizing of vagi produces only slightly greater lung contractions and that the vagi-lung muscle reacts to electrical stimulation like an ordinary nerve-muscle preparation. Time, 5 seconds.

These lung contractions in the snake appear to us too feeble to be of any value in the respiratory exchange of the lung.

2. *Lung reflexes.* Reflex lung contractions can be induced by the stimulation of the central end of one vagus, the other vagus being intact, and by stimulation of the nares, the cornea and the skin of the head region (fig. 22, *B*). The lung contraction following stimulation of the central end of one vagus is invariably preceded by an act of respiration, that is, a discharge from the respiratory center. The other reflex lung contractions may appear without being preceded by attempts at external respiration.

3. Rôle of the pulmonary vagi. Section of both vagi abolishes the lung contractions associated with the acts of external respiration. Stimulation of the peripheral end of either vagus causes contractions of the lung. These lung contractions appear to be confined to the upper third of the lung, that is, to the alveolated part or lung proper. It is a singular fact that, with one exception, all the paired lung reptilia thus far investigated, the lung motor action of the vagi is strictly unilateral, while in this species of snake both vagi act on the one lung. And it appears that the one-lunged condition of most snakes is due not to fusion of the original pair but to atrophy of one of the pair.

The lung motor fibers in the vagi of this snake can be stimulated with single induction shocks more readily than in the turtle. There is also less evidence of a tendency to an all-or-none motor response in the snake lung. In fact so far as our experiments go the vagus lung musculature behaves much like an ordinary nerve preparation.

Lung inhibitions from stimulation of the peripheral vagi were never observed. Destruction of the brain or section of both vagi neither decreased or increased the peripheral lung tonus. The lung vagi motor mechanism is either not in tonic activity or our method of preparing the animal abolishes this tonic activity through central "shock."

While our work on the snake was limited to eleven rather small specimens, the results indicate the essential parallel of the lung motor physiology in the snake and the turtle, namely, the vagi motor control and lung reflexes from the head origin of the animals. We have never observed a peripheral lung motor mechanism in the snake after section of the vagi.

THE DIRECT INFLUENCE OF THE SYMPATHETICS ON THE LUNG MOTOR TISSUES

Jackson and Pelz (7) have published tracings apparently showing strong inhibition of the lung tonus on stimulation of the central end of the cervical sympathetic nerve. These investigators also state that strong stimulation of this nerve may induce lung contractions of greater amplitude than that caused by stimulation of the peripheral end of the vagus. We have made great efforts to verify the results of Jackson and Pelz, particularly in view of the rôle which such inhibitory efferents may play in the lung reflexes.

We have been unable to follow nerve branches from the cervical sympathetic into the lung as figured by Jackson and Pelz. The sympathetic pulmonary branches described by them appear identical with the motor fibers from the brachial plexus to the striated muscle attached to the anterior and dorsolateral wall of the lung.

Stimulation of the central end of the cut cervical sympathetic may induce respiratory movements followed by lung contractions, provided the spinal cord and the vagi are intact. Or if the cervical sympathetic trunk is stimulated near its course past the brachial plexus, contractions of the striated lung muscle, previously referred to, may be produced by an escape of current to its motor fibers. These are the only motor effects on the lung that we have seen following the stimulation of the central end of the cervical sympathetic nerve, and neither are due to stimulation of sympathetic efferents.

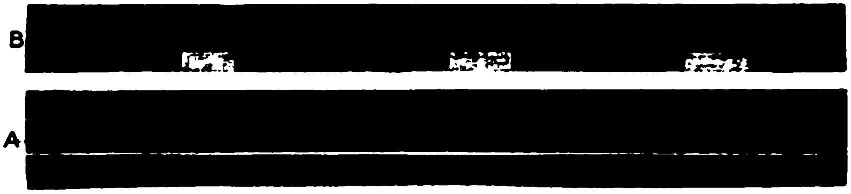

Fig. 23. Tambour tracings of the intrapulmonic pressure in the turtle. Animals decerebrated, fixed dorsal side down, plastron removed; stomach, liver and heart excised; vagi sectioned. Cannula in bronchus. *A:* Tracing showing a tonus rhythm in the lung independent of the vagi-medulla motor mechanism. *B:* Signal indicates stimulation of the central end of the cervical sympathetic nerve, showing slight inhibition (possibly reflex) of the peripheral lung tonus rhythm, developed after vagus section. Time, 5 second interval.

If the course of the sympathetic fibers to the lung in the reptilia is the same as in the amphibia and the mammals, these fibers should join the vagi in the neck or thorax and reach the lung via the pulmonary fibers of the vagi. We have stimulated the peripheral end of the cervical sympathetic nerve before its union with the vagus in very many preparations without any effect on the lung motor mechanism.

In one preparation only did we note any *inhibitory* effect on the lung from stimulation of the central end of the cervical sympathetic nerve. This preparation was decerebrated, fixed on the dorsal side, plastron removed, and eviscerated according to the method of Jackson and Pelz. A peripheral tonus rhythm appeared in the lung after section of the vagi. Tetanization (weak and strong) of the central end of the cervical sympathetic nerve induced some inhibition of this tonus

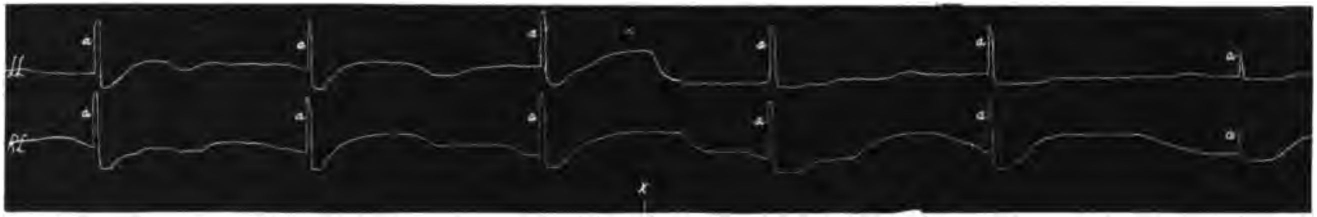

Fig. 24. Water manometer tracing of the intrapulmonic pressure in the turtle. Animal decerebrated, fixed dorsal side down; plastron removed; heart, liver, stomach and intestines excised; striated muscle on the anterior end of lung and its nerve connections left intact. *LL:* left lung; *RL:* right lung; *a*, spontaneous contraction (normal expiration) of the striated expiratory muscle on the surface of the lung, followed, during the respiratory pause, by prolonged tonus contractions of the lung musculature (asphyxia); *x*, section of the left vagus in the neck followed by cessation of the lung contractions, the respiratory contraction of the striated muscle on the lung persisting.

rhythm (fig. 23, *B*). Reflexes through the spinal cord were not excluded in this experiment.

The actual presence of sympathetic efferents to the reptilian lung is, therefore, an open question.

SUMMARY

1. Under normal conditions the external respiratory act inhibits the lung tonus by central action, and is followed by a contraction of the lung during the respiratory pause. These lung contractions are of central origin, the peripheral motor path being the vagi nerves (confirming Kahn and François-Franck).

2. The medullary center controlling the lung tonus and the lung contractions is not identical with the respiratory center, although normally associated with it in function in such a way that discharge from the respiratory center first depresses and then stimulates the lung motor center. The two centers are influenced in the same direction by asphyxial states, the lungs being put in a condition of incomplete tetanus by asphyxia, but one center may act, automatically or reflexly, without the other; and the lung motor centers are more profoundly influenced by afferent depressor nervous impulses (traumatic "shock").

3. The lung contraction developed during the respiratory pause is not a reflex depending on the muscular and other movements in the external respiratory act, as they persist after complete curarization, transection of the spinal cord in the neck, pithing the cord, or complete isolation of the lungs, save for the pulmonary vagi and blood vessels.

4. After section of the vagi a feeble tonus rhythm may appear in the lungs, but the strong contractions associated with external respiration are permanently abolished. Stimulation of the peripheral end of the vagi causes contractions and tetanus of the lungs, confirming the original observation of Bert. This vagus-lung action is unilateral. Stimulation of the peripheral vagus reveals no inhibitory action on the lung motor mechanism. Stimulation of the optic lobes or the medulla, the vagi being intact, causes lung contractions or incomplete lung tetanus (confirming Coombs). The lung tetanus is less complete than on direct stimulation of the peripheral end of the vagi, thus showing a central refractory state.

5. The physiological relation of the respiratory and the lung motor centers have a complete parallel in the relations of the activity of the swallowing center to the medullary mechanism controlling esophageal peristalsis. The action of the motor fibers of the vagi on the lung motor

tissues appears to be more direct and less complicated than the motor action of the vagi on the stomach, although there is some evidence of a tendency to an all-or-none response and rhythm in case of weak tetanization of the peripheral ends of the vagi even in the case of the lungs.

6. Inhibition as a primary act on the lung motor center in the medulla is produced by trauma to the vagi, stimulation of the central end of the inferior laryngeal nerve, and by strong stimulation of the central end of the pulmonary vagus. Trauma to the body causes a profound depression ("shock") of the lung motor center following an initial stimulation.

7. Reflex lung contractions or incomplete lung tetanus can be induced from stimulation of the sensory nerves of the respiratory tract (excepting the inferior laryngeal), that is by inflation, by deflation, or mechanical pressure on the lung of the opposite side, by weak tetanization of the pulmonary afferents, mechanical stimulation of the larynx and the nares. It is thus clear that the normal respiratory act by lung inflation and deflation will by itself induce a lung contraction reflexly. This afferent component from the lung is not necessary for the discharge of the lung motor center associated with the respiratory rhythm, but may act as a cumulative factor. The lung contractions and lung tetanus induced reflexly from mechanical stimulation of the posterior nares are particularly striking.

8. Mechanical and electrical stimulation of the alimentary tract (esophagus, gastric vagi, large and small intestine, rectum, cloaca) and the genito-urinary tract (urinary bladder, ureters, penis, prepuce, testes) induces reflex lung contractions or lung tetanus, the lung tetanus caused by stimulation of the cloaca, rectum and urinary bladder being particularly marked. Reflex lung contraction can also be evoked from stimulation of the gall bladder and the spleen.

9. Stimulation of the central ends of the visceral sympathetic nerves causes reflex lung contractions.

10. Stimulation (mechanical or electrical) of the cutaneous nerves, the cornea and the central end of the sciatic or brachial nerves induces reflex lung contractions or lung tetanus, depending on the strength of the stimulation.

11. The conclusions in regard to lung motor rhythm, its central and peripheral control, and the lung motor reflexes evoked from the head and neck region of the animal, apply both to the turtle and the snake. The lung reflexes from the viscera below the neck were not studied in the snake.

12. It would thus appear that the predominant reflex control of the lungs in these animals, at least under our experimental condition, is motor, thus differing entirely from the amphibia, where the predominating control (automatic and reflex) of the lung motor mechanism is inhibitory.

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STUDIES OF THE RESPIRATORY MECHANISM IN CARDIAC DYSPNEA

I. THE LOW ALVEOLAR CARBON DIOXIDE OF CARDIAC DYSPNEA

JOHN P. PETERS, JR. AND DAVID P. BARR

From the Russell Sage Institute of Pathology, in affiliation with the Second Medical Division, Bellevue Hospital and the Department of Medicine, Cornell University Medical College

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It has been shown by many observers, working with various methods, Beddard and Pembrey (1), Porges, Leimdoerfer and Markovici (2), Peabody (3), Pearce (4)—that the alveolar carbon dioxide tension is usually found to be low in cardiac dyspnea. The causes and meaning of this phenomenon have never been clear. In cardiac dyspnea, in contradistinction to most conditions that are associated with a low alveolar CO_2, no proportionate reduction of the alkaline reserve of the blood has been found.

In 1917 one of us (5) studied the relation of the carbon dioxide tension of alveolar air to the bicarbonate concentration of venous plasma. A low alveolar CO_2-tension did not prove to be a constant characteristic of cardiac dyspnea. The alveolar CO_2 was sometimes normal. But it was always lower than normal in relation to the alkaline reserve of the blood as determined by the Van Slyke method. Van Slyke (6) found that in normal subjects the alveolar CO_2-tension maintained a fairly constant relation to the plasma bicarbonate. If the milligrams of CO_2 chemically bound in 1 cc. of plasma is multiplied by the constant 35, the result will be found to agree with the alveolar CO_2-tension (Haldane) expressed in mm. Hg. with about a 10 per cent variation. That is:

$$(\text{Alveolar } CO_2 \text{ in mm. Hg.}) \div (\text{mgm. } CO_2 \text{ chemically bound by 1 cc. of plasma} \times 35) = 100 \pm 10.$$

This observation we corroborated. That such a ratio should obtain in normal resting subjects seems reasonable. To a certain extent it must represent the $H_2CO_3/NaHCO_3$ ratio, if the alveolar CO_2 can be assumed to be a measure of the arterial CO_2-tension and the bicarbonates of venous plasma an indication of the bicarbonates of whole blood.

In most normal resting subjects such assumptions will give rise to no serious errors. The difference between the carbon dioxide content of the arterial and the venous blood is not large and is comparatively constant. In pathological conditions which interfere with the general circulation or the ventilation of the blood in the lungs, a disturbance of these factors and consequently of the ratio may be expected.

In normal resting subjects the alveolar CO_2/plasma bicarbonate ratio varied between 0.90 and 1.10. In cardiac decompensation with dyspnea and in some very advanced pulmonary conditions we obtained ratios consistently below 0.85. To draw any definite conclusions as to the cause of the phenomenon was impossible. We suggested that it might be an expression of some defect of the normal mechanism for the elimination of carbon dioxide and pointed out the possibility of connecting this with the pulmonary changes which had been demonstrated by Siebeck (7), Peabody (8) and others.

Before any interpretation of the low alveolar CO_2[1] of cardiac decompensation is attempted it is obviously necessary to ascertain whether the method employed is applicable and whether the results obtained can be said to represent the true alveolar tension. The preliminary work was done with the Fridericia pipette (9). This has given rise to some criticism. Repetition with a more orthodox Haldane method has given substantially the same results. Although Pearce (4) has criticised the use of the Haldane method, observations by his own method are substantially in agreement with ours.

Siebeck (7), after a careful study of the respiratory mechanism in cardiac insufficiency, came to the conclusion that all determinations of the alveolar CO_2 were useless in this condition. According to Siebeck (7) the alveolar aeration in cardiac dyspnea is very imperfect, in consequence of which the expiratory air contains an excess of unchanged inspiratory air. Apparently he means that attempts to deduce the arterial CO_2-tension from the values obtained by the Haldane alveolar method are unwarranted. But it is perfectly possible to consider the alveolar CO_2 on its own merits as a functional entity without any deductions about arterial CO_2-tension. However well grounded the anatomical conceptions of Haldane with regard to the origin of the alveolar air in the normal lung may be, it is quite conceivable that in pathological or physiological disturbances, conditions may be so altered that any anatomical term loses its original significance. Here a functional conception proves of greater value.

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[1] Although the reduction of the alveolar CO_2-tension is not absolutely constant, few patients fail to show it. In these few the carbon dioxide capacity of the plasma is distinctly high, while in most instances it is at or slightly below the normal level. It seems proper and simpler, for this reason, to speak of the low alveolar CO_2 of cardiac dyspnea.

A normal expiration may be divided into two parts. The first part is practically useless from the standpoint of respiration; it merely serves to empty the dead space (the nose, mouth, pharynx, larynx, trachea and bronchi) of the room air with which it was filled by the last inspiration. In normal resting subjects the volume of this dead space is fairly constant, varying about an average of 130 cc. Behind this lies a mass of air of nearly constant gaseous composition throughout its whole extent and in close contact with the blood in the pulmonary circulation. This is the air which serves for the effective exchange of gases between the blood and the outside air, and it is a sample of this air that the Haldane method attempts to obtain.

The first object of these studies was to find out whether samples obtained by the Haldane method represented the effective respiratory air. We have employed other methods in order to determine whether they give results in agreement with the Haldane values.

Our studies have been confined to three methods: the Haldane (10), the Plesch (11) and the Henderson (12) venous CO_2. Pearce's (13) method has been omitted because its use in severe dyspnea presents considerable difficulties and also because it demands an amount of apparatus that renders it less adaptable as an ordinary clinical procedure.

THE HALDANE METHOD

The Haldane technique for obtaining alveolar specimens, although criticised for various reasons, is still considered the standard technique for the determination of the arterial CO_2-tension in normal subjects. It has not been generally adopted for clinical studies because of a popular opinion that it is difficult or impossible to obtain good samples in any but highly trained subjects. This we have not found to be the case. We are inclined to believe the observer needs more training than the subject.

In these studies we have adhered in all essential respects to the orthodox Haldane technique, introducing only slight modifications which tend to diminish subjective errors. One of these is by no means original and consists of the introduction of a three-way brass stop-cock designed by Mr. G. F. Soderstrom and shown in figure 1. This makes it unnecessary for the patient to close the tube with his tongue. Furthermore, no attempt has been made to collect inspiratory and expiratory specimens, as Haldane advises. Instead, samples have been obtained from the end of a forced expiration begun as soon as possible after the completion of a normal inspiration. When the respirations are rapid, as they are in cardiac dyspnea, it is almost impossible to turn the valve at the proper moment to differentiate inspiratory and expiratory alveolar air with any degree of certainty. The propriety of using an expiratory specimen also seems questionable. The prolongation of expiration during dyspnea may be equivalent to holding the breath for the duration of an extra respiration. We have tried to make the subjects force the air out rapidly enough so that the expiration is not considerably longer than a normal one. The specimens as obtained by this method are not all from expirations initiated at the same

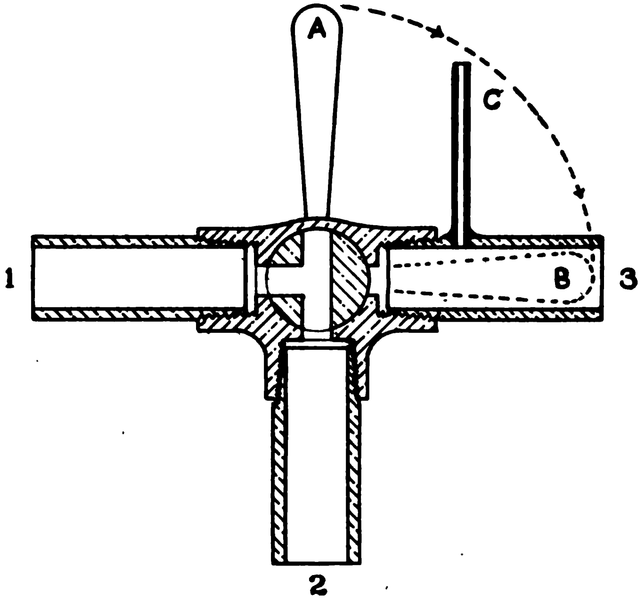

Fig. 1. Three-way stop-cock. *1*, tube for attachment of mouth-piece; *2*, tube leading to spirometer or left open to outside air; *3*, tube for attachment of Haldane tube, with side-arm *C*, to connect with gas sampling tube.

point in the respiratory cycle, but at various points in the expiratory phase between full inflation and deflation of the lungs.

In detail the procedure is as follows: The rubber mouth-piece with the stop-cock and tube in place is first put in the patient's mouth with the stop-cock open to the air. He is encouraged to breathe through it to convince him that it will not "shut off his breath." After he has gained sufficient confidence the tube is removed from his mouth and a simple spring nose-clip applied to his nose. When he is sure he can breathe through his mouth alone without difficulty the mouth-piece is replaced in his mouth. He is then instructed how to deliver a sample on command and without altering his normal respirations. The sampling tube is then attached to the side-arm of the stop-cock. The operator stands at the right of the bed supporting the tube with his left hand, with his right on the handle of the stop-cock. When he feels that the breathing is normal and regular he tries to accustom himself to its rhythm, so that he may give the signal at the right moment. The signal is given and the stop-cock turned at the same moment. At the end of the forced expiration the cock is turned to the outside air again before the subject has had time to gasp for breath. The operator soon learns to know whether there has been a subjective error in the technique on his own part or that of the patient.

From an inexperienced subject we are accustomed to take several specimens. Although some of the first will occasionally be obviously too low, it is generally possible to get good agreement on the third or fourth attempt in even the most obtuse subject, as may be seen in tables 1 and 2. In a total of 48 observations on 25 patients, involving the collection and analysis of 127 samples, all but four patients gave duplicate samples that varied by 0.4 per cent or less, an accuracy that compares favorably with that found in trained subjects. Patients with cardiac dyspnea proved no exception to this rule. Three of the four patients (A. R., J. J. F. and D. W.) who gave unsatisfactory results on the basis of this criterion, exhibited Cheyne-Stokes breathing. The fourth (P. O. S.) was very ill, somewhat irrational and showed a slight respiratory irregularity.

The results obtained by the Haldane method are in accordance with those previously obtained with the Fridericia tube. The values are comparatively low and much lower than should be expected from the level of plasma bicarbonates. Two obvious criticisms of the method might be made:

1. That the volume of the expiration is too small to clear the patient's dead space.

TABLE 1

Variations in duplicate Haldane specimens in subjects without respiratory or cardiac disorders

SUBJECT AND CONDITION	ALVEOLAR CO_2	MAXIMUM OBSERVED VARIATION	MAXIMUM ACCEPTED VARIATION*	AVERAGE ALVEOLAR CO_2
	per cent			*mm.*
D. P. B., normal	6.16 5.94	0.22	0.22	43.1
	5.25 5.31	0.06	0.06	37.4
	5.72 5.74	0.02	0.02	40.6
	5.39 5.26	0.13	0.13	37.9
	5.16 5.40	0.24	0.24	37.6
	5.39 5.57	0.18	0.18	39.0
	5.60 5.60	0.00	0.00	38.7
	5.39 5.60	0.21	0.21	39.2
	5.45 5.50	0.05	0.05	38.8
	5.62 5.51	0.11	0.11	39.1
	5.32 5.24 5.58	0.34	0.34	38.6
J. P., normal	(5.28) 5.64 5.74 5.45	0.46	0.29	39.9
	5.94 5.85	0.09	0.09	41.6

TABLE 1—*Concluded*

SUBJECT AND CONDITION	ALVEOLAR CO_2	MAXIMUM OBSERVED VARIATION	MAXIMUM ACCEPTED VARIATION*	AVERAGE ALVEOLAR CO_2
	per cent			*mm.*
J. P., normal	5.88	0.26	0.26	40.5
	5.62			
	5.35	0.15	0.15	37.5
	5.20			
Capt., normal	6.24	0.07	0.07	44.4
	6.31			
W. S. M., normal	4.70	0.12	0.12	34.8
	4.82			
P. K., gastric neurosis	5.88	0.25	0.25	41.2
	5.61			
	5.86			
Jno. K., diabetes mellitus	4.24	0.16	0.16	30.1
	4.19			
	4.35			
	4.07	0.05	0.05	28.4
	4.06			
	4.02			
C. P., pernicious anemia	3.98	0.11	0.11	28.2
	3.91			
	4.02			
H. R., polycythemia	4.94	0.32	0.32	34.7
	5.11			
	4.79			

* Values in parenthesis in this and the following table have been discarded for the most part on the basis of internal evidence, only.

2. That the low values are produced by subjective errors; preliminary forced inspirations or gasping at the end of expiration.

To rule these out we attached the Haldane tube to a Tissot spirometer which was equipped with a calibrated recording device which will be described later. By this means we were enabled to obtain a graphic record of the respiration during the entire time that the stopcock was turned, and could at the same time measure the volume of the

TABLE 2

Variations in duplicate Haldane specimens with values for alveolar CO_2 and plasma bicarbonates in patients with cardiac disease

NAME AND DIAGNOSIS	ALVEOLAR CO_2	MAXIMUM OBSERVED VARIATION	MAXIMUM ACCEPTED VARIATION	AVERAGE ALVEOLAR CO_2	ALVEOLAR CO_2 CALCULATED FROM PLASMA BICARBONATE	DIFFERENCE BETWEEN CALCULATED AND OBSERVED ALVEOLAR CO_2	REMARKS
	per cent			*mm.*	*mm.*	*mm.*	
J. W., cardionephritic	4.71 4.75 4.76	0.05	0.05	34.6	39.7	5.1	Hypertension. No dyspnea, hyperpnea nor other signs of decompensation
J. A., cardionephritic	(5.21) 6.01 5.76 5.99	0.80	0.25	42.3	49.7	7.4	Hypertension, no dyspnea, cyanosis nor other signs of decompensation at rest
	4.38 3.98 4.25	0.40	0.40	30.0	41.5	11.5	Slight dyspnea and cyanosis. Lungs show râles at right base
A. J., shell shock	4.59 4.43 (4.03)	0.56	0.16	32.5	46.8	14.3	Effort syndrome. No organic cardiac nor pulmonary condition. Discrepancy probably due to over-ventilation
	4.86 4.78	0.08	0.08	33.6			

J. M. L., chronic cardiac	5.43 5.39	0.04	0.04	38.5			No symptoms nor signs of decompensation. Lungs clear
	5.11 5.35	0.24	0.24	37.2			
A. R., cardionephritic	3.96 4.23 4.78 4.21	0.82	0.82	30.6	31.9	1.3	Hypertension, signs of chronic uremia. Variations in alveolar samples probably due to Cheyne-Stokes breathing
R. W., chronic cardiac	5.69 5.90 5.67 5.78	0.23	0.23	41.0			No dyspnea, cyanosis nor other signs of decompensation
	5.79 6.12	0.33	0.33	42.4			
	6.10 6.22 6.52	0.42	0.42	44.7			
J. C., chronic cardiac	4.65 4.43 4.39	0.26	0.26	32.0			No dyspnea, cyanosis nor other signs of decompensation while at rest. The low alveolar values obtained in the first observation probably due to overventilation
	5.09 5.33 5.37 5.38	0.29	0.29	38.5	43.9	5.4	

TABLE 2—*Continued*

NAME AND DIAGNOSIS	ALVEOLAR CO_2	MAXIMUM OBSERVED VARIATION	MAXIMUM ACCEPTED VARIATION	AVERAGE ALVEOLAR CO_2	ALVEOLAR CO_2 CALCULATED FROM PLASMA BICARBONATE	DIFFERENCE BETWEEN CALCULATED AND OBSERVED ALVEOLAR CO_2	REMARKS
	per cent			*mm.*	*mm.*	*mm.*	
J. J. F., chronic cardiac	4.62 5.08 5.12 4.43	0.69	0.69	34.2	45.5	11.3	
D. W., cardionephritic	(3.08) 4.07 (3.22) 3.59	0.99	0.48	27.3	30.2	2.9	Aortic regurgitation with hypertension. Dyspnea and orthopnea with Cheyne-Stokes breathing. Slightly irrational. Great variations largely due to inadequate coöperation. Low values eliminated by graphic records. Dyspnea probably due to acidosis and not to cardiac disease
P. O. S., chronic cardiac	5.85 5.06 5.27 5.53	0.79	0.79	40.1	52.3	12.2	Extreme decompensation, dyspnea, orthopnea, and Cheyne-Stokes breathing
J. B., chronic cardiac	4.55 4.45	0.10	0.10	32.1	44.8	12.7	Moderate hyperpnea, some cyanosis. Slight edema. Lungs clear. No subjective dyspnea
	4.04 4.18	0.14	0.14	29.3			
	4.21 4.01	0.20	0.20	29.3			

W. W., cardionephritic	3.84 4.15	0.31	0.31	28.5			Moderate dyspnea and orthopnea while at rest. Some signs of fluid in right pleural cavity. Râles over base of left lung
	4.08 4.00 3.97 3.98	0.11	0.11	28.6	43.7	15.1	
J. D. B., chronic cardiac	3.11 3.55	0.06	0.06	25.4			Considerable dyspnea and hyperpnea and orthopnea. Moderate cyanosis
	3.35 3.69	0.34	0.34	25.3	41.7	16.4	
	3.85 3.82 3.90 3.89	0.08	0.08	27.6	46.6	19.0	
	4.51 4.62 4.64 4.34	0.30	0.30	32.2			
J. R., chronic cardiac	4.59 4.56 4.64	0.08	0.08	32.8	48.0	15.2	Severe dyspnea, orthopnea, edema, right hydrothorax
C. D., chronic cardiac	5.10 4.89	0.21	0.21	35.9	46.9	11.0	Severe dyspnea and orthopnea, slight cyanosis. Slight dulness at base of right lung
	4.43 4.75 4.53	0.32	0.32	32.5			

TABLE 2—*Concluded*

NAME AND DIAGNOSIS	ALVEOLAR CO_2	MAXIMUM OBSERVED VARIATION	MAXIMUM ACCEPTED VARIATION	AVERAGE ALVEOLAR CO_2	ALVEOLAR CO_2 CALCULATED FROM PLASMA BICARBONATE	DIFFERENCE BETWEEN CALCULATED AND OBSERVED ALVEOLAR CO_2	REMARKS
	per cent			*mm.*	*mm.*	*mm.*	
Jos. C., cardionephritic	5.36 (4.95) 5.53 5.43	0.58	0.17	38.7	47.8	9.1	Hypertension, auricular fibrillation, marked dyspnea and orthopnea. Some cyanosis. Deformity of right chest
	5.97 5.75 6.06	0.31	0.31	42.2	49.7	7.5	
	(5.78) 6.22 6.60 6.31 6.18	0.82	0.42	44.9	52.2	7.3	
S. I., chronic cardiac	4.01 3.65 3.91	0.36	0.36	27.4	47.6	20.2	Extreme dyspnea, orthopnea, cyanosis and edema, right hydrothorax, râles over remainder of lungs
D. O. C., chronic cardiac	4.21 4.12	0.09	0.09	29.7	40.9	11.2	Considerable cyanosis, orthopnea, and dyspnea. Signs of fluid in right pleural cavity
	3.61 3.83	0.22	0.22	26.5			Cyanosis and dyspnea more marked

D. O. C., chronic cardiac	3.61 3.99 3.67	0.38	0.38	26.7			Condition unchanged
	3.70 3.92 (4.43) 3.56	0.87	0.36	26.6	38.2	11.6	Pleural fluid has increased. Dyspnea more marked
	3.97 3.61	0.36	0.36	23.6			Fluid in both pleural cavities
	3.52 3.82 3.70	0.30	0.30	26.2			Condition unchanged
J. M., chronic cardiac	3.52			25.0	40.9	15.9	Extreme cyanosis. Moderate dyspnea, considerable hyperpnea, right hydrothorax
	4.87 5.03	0.16	0.16	34.8	52.0	17.2	No dyspnea, moderate hyperpnea, cyanosis still marked. Hydrothorax diminished
J. K., chronic cardiac	3.26 3.13 3.03 3.03	0.23	0.23	22.0	37.8	15.8	Extreme cyanosis, slight dyspnea, considerable hyperpnea, few râles scattered over both lungs
	4.24 4.86 4.56	0.62	0.62	32.5			Dyspnea gone, cyanosis slight, still some hyperpnea. Lungs clear
C. C., chronic cardiac	3.95 3.68 3.91	0.27	0.27	27.4			Marked cyanosis. Little dyspnea. No orthopnea. Considerable hyperpnea

TABLE 3

Relation of alveolar CO_2 to volume of expiration

SUBJECT	VOLUME OF EXPIRATION	ALVEOLAR CO_2
	cc.	*per cent*
J. C., chronic cardiac compensated	1016	5.09
	823	5.33
	807	5.37
	790	5.38
W W., chronic cardiac decompensated	797	4.08
	864	4.00
	615	3.97
	665	4.35
	385	4.32
R. W., chronic cardiac compensated	710	5.90
	452	5.67
	645	5.78
	758	6.10
	651	6.22
	516	6.52
J. D. B., chronic cardiac decompensated	1081	4.51
	1290	4.62
	758	4.64
	986	4.34
A. R., cardionephritic. Periodic breathing	505	3.96
	535	4.23
	430	4.78
	505	4.21
D. W.,* cardionephritic. Periodic breathing	(758	3.08)
	677	4.07
	(629	3.22)
	952	3.59

* See figure 2.

expiration. Table 3 shows the values obtained from six experiments done in this way. From the first five experiments it is evident that the expiratory volume is sufficient to clear completely any but an enormously increased dead space. If the dead space were increased to this degree, however, the alveolar CO_2-tension should vary with the expiratory volume. This relation does not obtain. In only one instance, the second observation on R. W., is any such relation even suggested, and in this case the total variation is only 0.23 per cent.

In none of these cases is there any indication in the graphic records of inspiratory activity during the time that the stop-cock was open. That the method is capable of showing such subjective errors is demonstrated in the record of D. W. (fig. 2). This patient presented symptoms of uremia with Cheyne-Stokes respiration. His tracings show that the low values obtained from the first and third samples were due to inspirations while the valve was open and before the samples had been withdrawn. The discrepancy in the two remaining values seems to depend on the fact that one was obtained during an apneic, the other during a dyspneic period.

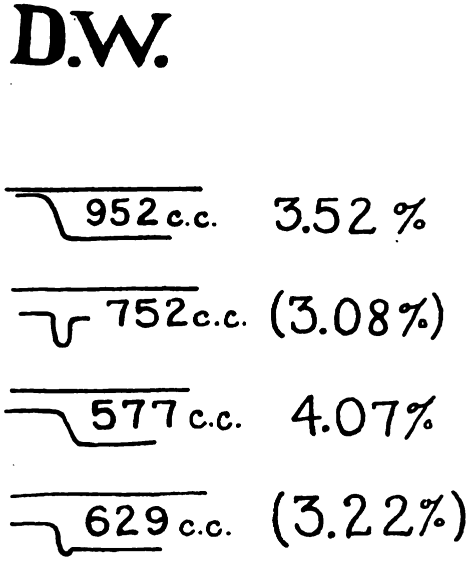

Fig. 2

The low alveolar CO_2 is not due to subjective errors on the part of the patient nor to any considerable increase in the volume of the dead space.

The alveolar CO_2 after rebreathing. One of the most striking characteristics of decompensated cardiac patients is the intolerance to an increase of CO_2 in the inspired air (14).

It has been shown by Haldane (15) and others that when a person rebreathes air the alveolar CO_2-tension and the ventilation increase as the CO_2 in the inspired air rises. When the alveolar CO_2-tension has risen to a certain point the patient becomes extremely dyspneic and uncomfortable. This we may call the point of intolerance to CO_2. If the alveolar CO_2 be low in cardiac dyspnea at rest it seems reasonable to suppose that it should also be relatively low after rebreathing to the point of intolerance.

For the rebreathing experiments a 100-liter Tissot spirometer, accurately balanced and calibrated at all levels, was employed. To separate the inspired from the expired air a two-way T-valve of the Douglas type was used. Between the mouth piece and this T-valve was interposed the three-way brass stop-cock described above. The opening with the side-arm was, as usual, fitted to a long, wide-bore rubber tube for the collection of alveolar specimens. The other arm was connected with the T-valve. (The total instrumental dead space was about 50 cc.) In this way it was possible to collect specimens of alveolar air during the course of a rebreathing experiment by merely turning the stop-cock during expiration and directing the patient to breathe out forcibly. The inspiratory air was withdrawn from the spirometer through the opening in the top usually employed for the insertion of the thermometer. Samples of the inspiratory air were drawn from this tube near the T-valve through a small rubber side arm. Graphic records of the respirations were obtained by means of a pen attached to the counter-weight of the spirometer. As it moved in a vertical plane only, direct calibration was possible. Simultaneous time records were obtained. The apparatus is represented diagrammatically in figure 3.

The spirometer was filled with 20 liters of room air for each experiment. A larger amount was found to prolong the experiment unduly and to introduce a considerable element of fatigue. Smaller amounts rendered the succession of events too rapid to permit the introduction of all the necessary procedures and accurate analysis of the results obtained.

The minute volume attained in the rebreathing experiments was calculated from the average tidal air and the respiratory rate during the last half-minute of the experiment, except in one or two cases where the limit of tolerance was reached so rapidly that it was necessary to use a shorter terminal period for purposes of calculation. Of course, assuming that the minute volume increases continuously throughout

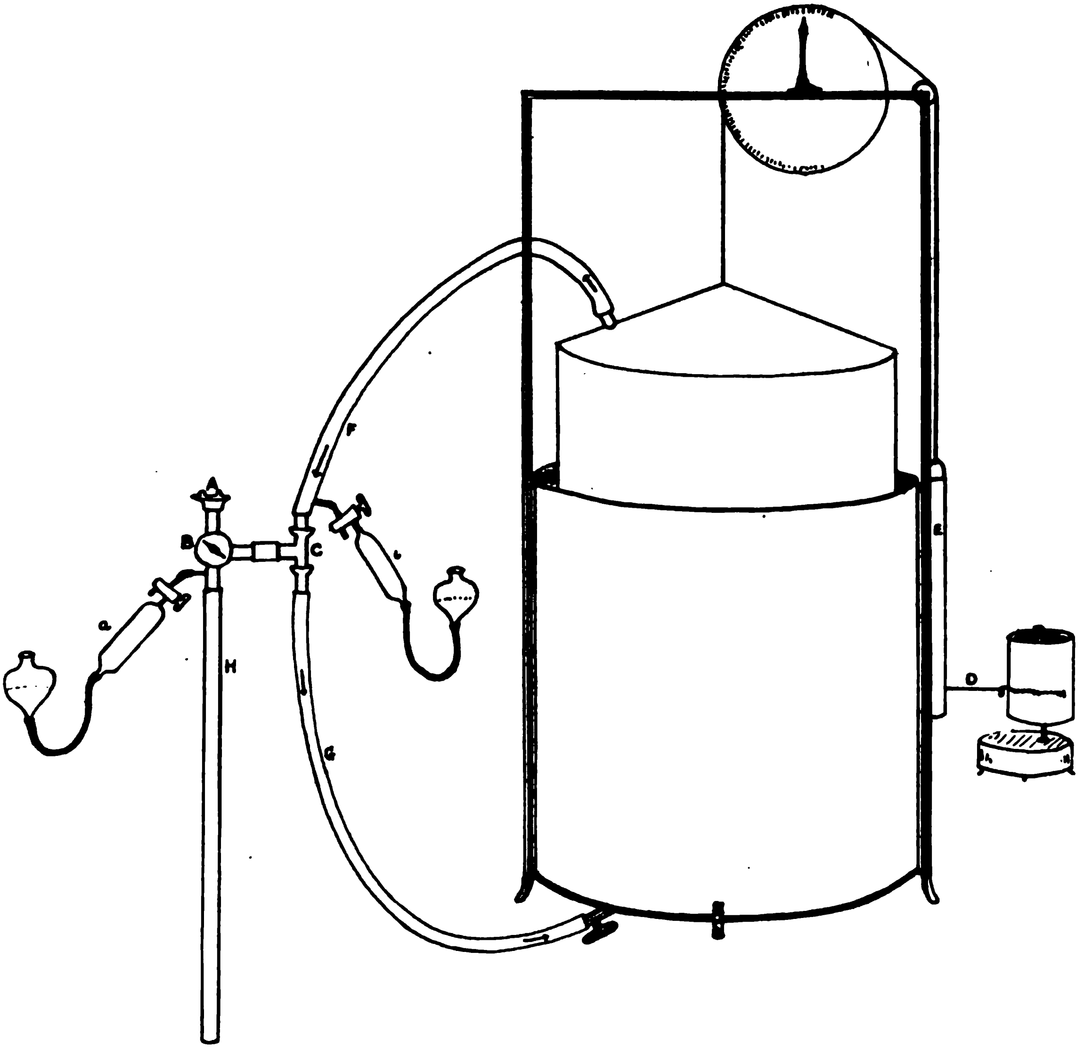

Fig. 3. *A*, mouth-piece; *B*, 3-way stop-cock; *C*, Douglass valve; *F*, inspiratory tube; *G*, expiratory tube; *E*, counterweight of spirometer; *D*, recording pen; *H*, Haldane tube; *a*, sampling tube for alveolar air; *i*, sampling tube for inspiratory air.

the course of the experiment, the values obtained in this way must be somewhat too low. On the other hand, the respirations never become absolutely regular, and calculations based on too small a number will be subject to considerable error. In rebreathing so large a volume as 20 liters the respiratory increase is sufficiently gradual to permit the use of the last half-minute, in the majority of instances, with the introduction of no significant error.

After a satisfactory determination of the resting alveolar CO_2 and minute volume had been made, a rebreathing experiment was begun. The patient was urged to continue as long as he felt able. When he had reached the limit of tolerance the valve of the three-way stop-cock was opened to the Haldane tube during an expiration. The patient was told to breathe out forcibly and the valve was again closed before the following inspiration. The patient was at once disconnected from the apparatus and the alveolar sample removed for analysis. The second operator at the same time removed a sample from the inspiratory tube.

Of course, the coördination and coöperation of the subject were necessary and there were opportunities for mistakes. Altogether eleven successful experiments were made on two normal subjects; one out of two was successful in a patient with a gastric neurosis; one in shell shock; three in two compensated cardiacs; and five out of seven attempted in four patients with cardiac decompensation. The results appear in summarized form in table 4.

A very striking difference is apparent at once between the decompensated cardiac cases and the others. The former were unable to tolerate as much CO_2 in the inspired air and their alveolar CO_2 did not rise to as high a level. This was due to no lack of will power on the part of the cardiacs because in one or two cases they continued rebreathing to the point of exhaustion, while none of the compensated subjects went beyond a state of moderate discomfort. The alveolar CO_2 at the point of intolerance is therefore lower in decompensated cardiacs than it is in normal persons.

With the exception of A. J., the shell shock patient, the height of the alveolar CO_2 at which intolerance was reached bore a general relation to the preliminary alveolar CO_2-tension. In this one case the low resting alveolar CO_2 was due to over-ventilation. This was clearly shown by the fact that his initial reaction to rebreathing was a reduction of the minute-volume, instead of an increase.

TABLE 4

The effect of rebreathing on the alveolar CO_2

NUMBER AND NAME	RESTING ALVEOLAR CO_2	AT END OF REBREATHING EXPERIMENT		DIAGNOSIS AND REMARKS
		Inspiratory air CO_2	Alveolar CO_2	
	per cent	*per cent*	*per cent*	
1. D. P. B.		8.36	8.52	Normal adult. Continued rebreathing to point of considerable discomfort
	6.05	8.01	8.16	
		7.93	8.21	To discomfort
	5.28	8.06	8.30	To discomfort
	5.73	7.84	7.95	To slight discomfort
		6.86	7.53	To slight discomfort
2. J. P.....		7.90	8.32	Normal adult. To considerable discomfort
		7.95	7.98	To extreme discomfort
		6.90	7.27	To discomfort
		6.86	7.05	To considerable discomfort
3. P K.....	5.78	6.61		Gastric neurosis
	5.50	6.07	6.85	To moderate discomfort. No exhaustion
4. T. deC..	5.50	5.90	7.30	Chronic cardiac valvular disease, compensated
	5.70	6.59	7.27	Rebreathed to point of moderate discomfort
5. J. M. L...	5.23	7.54	7.92	Chronic cardiac. Compensated. Continued rebreathing to point of considerable discomfort
6. A. J.....	4.51	6.26	6.30	Shell shock with effort syndrome. To moderate discomfort only
	4.72	6.30	6.89	
7. J. A.....	5.92	5.74	6.36	Chronic cardiac, valvular disease, compensated while at rest. Rebreathed to point of considerable discomfort with some exhaustion
		4.52	7.34	
8. J. B.....	4.50	3.70		Chronic cardiac valvular disease. Moderate dyspnea while at rest. In the first two experiments continued only to mild discomfort. In the last experiment considerable discomfort. Somewhat exhausted
	4.50	3.92	5.55	
	4.11	5.17	6.26	

TABLE 4—*Concluded*

NUMBER AND NAME	RESTING ALVEOLAR CO_2	AT END OF REBREATHING EXPERIMENT		DIAGNOSIS AND REMARKS
		Inspiratory air CO_2	Alveolar CO_2	
	per cent	*per cent*	*per cent*	
9. Jos. C....	5.93	3.88	6.68	Cardio-nephritic with cardiac decompensation. Considerable dyspnea and hyperpnea. Continued to point of considerable discomfort. Somewhat exhausted
10. C. D....	5.00	2.13	5.10	Chronic cardiac, valvular disease with marked decompensation. Continued to point of marked exhaustion
	4.57	2.89		
11. D. O. C.	4.17	2.94		Chronic cardiac, valvular disease with decompensation. Continued rebreathing to point of discomfort with some exhaustion
		2.53	5.72	

In cardiac dyspnea, then, the alveolar CO_2 is low at the point of intolerance. The relation of the alveolar CO_2 at this point to the resting alveolar CO_2 is similar to that found in normal persons. This is further evidence that the air in the lungs in cardiac dyspnea really presents a diminished CO_2-tension.

THE PLESCH METHOD

Modifications of the Plesch method of obtaining samples of alveolar air have been employed extensively in cardiac disease and other pathological conditions by numerous workers. Both Porges (2) and Peabody (3) used it for their studies on cardiac dyspnea. With this method, the subject rebreathes a limited volume of air, usually 600 to 1000 cc. a number of times, usually from 5 to 10, in a period of time usually between 20 and 30 seconds, which is supposed to be less than the duration of a single complete circulation of the blood. With normal resting subjects it gives values that lie a little higher than those obtained by the Haldane method and is assumed to represent the tension of CO_2 in the venous blood.

Usually the rate and depth of the respirations and the duration of rebreathing can be varied within rather wide limits with only a slight variation in the analyses obtained. This might reasonably be expected even if the theoretical basis of the method were entirely wrong. It has been shown that in normal subjects at rest, lung volume, carbon dioxid output, arterial CO_2-tension and venous CO_2-tension vary within narrow limits absolutely and in relation to one another.

The assumption that such an empirical method is equally applicable to the study of a condition in which all these factors are greatly disturbed, is hardly justifiable.

We have made a few observations on normal subjects and on patients with cardiac disease (see table 5) in which we tested the effect of varying the time and rate of rebreathing within certain limits. The time limits chosen were 25 and 35 seconds; the number of breaths 5 and 10. The apparatus used was essentially the same as that recommended by Peabody (16): a three-way brass stop-cock described above, which connected the subject either with the outside air or a rebreathing bag containing 1000 cc. of room air. The bore of the valve and tubing was 1.75 cm., the instrumental dead space, including the rubber mouthpiece, 30 cc. Rebreathing was always begun after a forced expiration and the patient was directed to make a maximum respiratory effort with each breath.

TABLE 5

Results of varying time and number of respirations on Plesch-alveolar CO_2

	NORMALS				J. M. L. COMPENSATED CARDIAC	DECOMPENSATED CARDIACS					
	D. P. B.			J. P.		S. I.	D. O. C.		W. W.	J. B.	
5 respirations in 25 seconds	6.17	6.04	5.24	6.29	6.08	4.48	4.85	5.41	4.70	5.03	4.82
10 respirations in 25 seconds	6.53	5.97	5.90	6.22	6.76	4.62	4.92	5.88	5.41	5.14	5.53
5 respirations in 35 seconds	6.43	6.35	5.89	6.34	6.90			5.99	5.67	5.63	5.81
10 respirations in 35 seconds	6.34	6.41	5.88	6.43	6.92	4.70		6.29	5.99	5.50	
Average value	6.37	6.19	5.73	6.32	6.67	4.60	4.89	5.89	5.44	5.33	5.17
Maximum variation	0.36	0.44	0.66	0.21	0.84	0.22	0.07	0.88	1.29	0.60	0.99
Haldane alveolar CO_2	5.28	5.33		5.61	5.41	3.86	3.72	3.75	4.34	4.11	4.11
Difference between Plesch and Haldane	1.09	0.86		0.71	1.26	0.74	1.17	1.99	1.10	1.22	1.06

In the case of J. P. the total difference obtained within the limits of variation of time and number of respirations employed was 0.21 per cent. In D. P. B. the differences were greater: from 0.36 to 0.66 per cent. Moreover, in one experiment on the latter (no. III) it appears that the duration of the experiment has less influence than the number of respirations. In the cardiac patients studied the differences were slightly larger, in one case 1.29 per cent. Again variations in the number of respirations appear almost as important as variations in time. One would be rather at a loss to know which values to accept under these conditions.

Of course 35 seconds and 10 breaths are both in excess of the limits usually employed. The objection may be raised that we have not limited the element of time and the number of breaths sufficiently. To us it seems essential to determine whether limitation of experimental factors produces an equivalent limitation of variation in all subjects. One is not justified in producing an apparent constancy in results by an arbitrary limitation of experimental variations.

THE HENDERSON-LAURENS METHOD

Y. Henderson (12) has recently published a method for the determination of the venous CO_2-tension. It does not differ in principle from previous methods, but demands less effort and intelligence on the part of the subject and is therefore more applicable to clinical studies. It depends on the principle of intermittent rebreathing. Laurens (17) has pointed out that it is necessary to regard certain precautions in rebreathing because diffusion of CO_2 is more perfect during respiratory motions than it is while the chest is motionless. Our technic has been modified, therefore, to meet his requirements.

The apparatus used was the same as that employed in the Plesch studies. The rebreathing bag was filled with about 2000 cc. of expiratory air. In most cases a second observation was made in which the bag was filled with a mixture of air with 6.5 to 10 per cent CO_2. While the patient was breathing room air quietly through the valve, he was ordered to give a maximum expiration. When his lungs were completely deflated the stop-cock was turned. He then filled his lungs with a mixture from the bag by a deep inspiration, retained the air in his lungs about 10 seconds and expired into the bag forcibly. The stop-cock was then turned to the room again and he resumed normal respirations. After a sufficient interval to permit the respirations to return to normal the same procedure was repeated. After each respiration the mixture in the bag was tested for CO_2. The rebreathing was continued until analyses after successive respirations showed that the CO_2-tension had reached a practically constant level. In normal subjects this level is reached after about 7 rebreathings and the level attained in rebreathing expired air and CO_2 rich mixtures is the same.

The results of these studies are shown in table 6. Not all of the experiments are entirely satisfactory; but a more or less constant level is reached in cardiac subjects as well as in normals.

There is a surprisingly close agreement between the venous alveolar values obtained by the Henderson-Laurens method and the average of the values obtained by the Plesch method. The application of the Plesch method, in the simple form usually employed, to the physiologic study of cardiac disease, seems hardly warranted. The limitation of the number of respirations and the duration of rebreathing is more or less arbitrary and based on the study of normal subjects only. The use of an average value obtained from a series of observations in which both time and number of respirations has been varied seems preferable. In normal and cardiac subjects values so obtained agree with those obtained by at least one other venous method.

The difference between the Henderson and the Haldane alveolar CO_2 is greater in subjects with cardiac dyspnea than in normal persons or cardiac patients without dyspnea. In spite of this the actual values found by the Henderson method in cardiac dyspnea are slightly lower than normal. This is again evidence that the carbon dioxide tension of the air in the lungs is reduced in cardiac dyspnea.

It is not possible to argue that the difference between the Henderson and the Haldane alveolar CO_2 represents the difference of CO_2-tension between arterial and venous blood. The Haldane values can not be interpreted as a measure of the arterial CO_2-tension until the criticisms of Siebeck have been answered. The only way to answer them is by the direct determination of the arterial CO_2-tension. The objections to the application of alveolar methods to the determination of the carbon dioxid tension of venous blood are even greater. Christiansen, Douglas and Haldane (18) have pointed out that direct determination of the venous carbon dioxid tension by these methods is impossible. The peculiar effect of oxygen on the carbon dioxid dissociation curve of the blood necessitates the introduction of a correction for the oxygen unsaturation of venous blood. An average correction can be applied in the case of normal persons without any considerable error. In

TABLE 6

Comparison of Henderson-Laurens with Haldane method and its application to cardiac dyspnea

SUBJECT AND CONDITION	REBREATHING EXPIRED AIR		REBREATHING CO_2-AIR MIXTURE			CALCULATED VENOUS ALVEOLAR CO_2	PLESCH ALVEOLAR CO_2	HALDANE ALVEOLAR CO_2	DIFFERENCE BETWEEN HENDERSON AND HALDANE	ALVEOLAR CO_2 CALCULATED FROM PLASMA BICARBONATE
	Number of rebreathings	CO_2 found in bag	Mixture rebreathed CO_2	Number of rebreathings	CO_2 found in bag					
		per cent	*per cent*		*per cent*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
D. P. B., normal	2	5.25				44.9	45.8	37.9	6.9	
	3	5.74								
	4	6.17								
	5	6.28								
	4	5.69				45.1	45.4	37.6	7.5	
	5	6.21								
	6	6.26								
	7	6.38								
			6.64	6	5.79	43.4		39.0	4.4	
	7	6.14		7	5.82					
	8	6.07		8	5.86					
	9	6.13		9	6.01					
			7.55	6	5.97	42.7		39.3	3.4	
	7	5.95		7	5.88					
	8	6.23		8	5.93					
	9	6.06								
	7	5.80	10.02	5	5.99	41.9		39.2	2.7	
	8	5.84		6	6.03					
	9	5.79		7	5.91					

J. P., normal	2	6.13				43.8	45.0	39.9	3.9	
	3	6.13								
	5	6.20								
			10.34	3	7.02	48.6				
				4	7.01					
	7	6.67		5	6.93					
	8	6.83		6	6.86					
	9	6.83		7	6.77					
	8	6.14	6.96	5	6.34	44.8				
	9	6.23		6	6.28					
	10	6.21		7	6.34					
			7.14	5	5.99	41.9		42.3	4.0	
	7	5.82		6	5.98					
	8	5.76		7	5.95					
J. A., chronic cardiac compensated	4	6.45				46.6		42.3	4.3	49.7
	5									
	6	6.53								
	7	6.52								
J. C., chronic cardiac compensated	7	5.59	10.0	7	6.53	44.9		37.7	7.2	
	8	5.75		8	6.45					
	9	6.00								
J. M. L., chronic cardiac compensated	3	6.25				48.4	47.5	38.5	9.9	
	4	6.66								
	5	6.74								
R. W., chronic cardiac compensated			7.64	7	6.44	47.8		42.4	5.4	
	8	6.44		8	6.72					
	9	6.52		9	6.72					

TABLE 6—*Concluded*

SUBJECT AND CONDITION	REBREATHING EXPIRED AIR		REBREATHING CO$_2$-AIR MIXTURE			CALCULATED VENOUS ALVEOLAR CO$_2$	PLESCH ALVEOLAR CO$_2$	HALDANE ALVEOLAR CO$_2$	DIFFERENCE BETWEEN HENDERSON AND HALDANE	ALVEOLAR CO$_2$ CALCULATED FROM PLASMA BICARBONATE
	Number of rebreathings	CO$_2$ found in bag	Mixture rebreathed CO$_2$	Number of rebreathings	CO$_2$ found in bag					
		per cent	*per cent*		*per cent*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
J. B., chronic cardiac decompensated	2	5.07				38.9	38.4	29.3	9.6	
	3	5.48								
	4	5.43								
	5	5.25								
Jos. C., chronic cardiac decompensated	4	6.11				45.2		38.7	6.5	47.8
	5	6.35								
	6	6.39								
	7	6.30								
	6	6.77	8.92	5	6.99	48.9		44.9	4.0	52.2
	7	6.34		6	7.06					
	8	6.40		7	6.95					
W. W., chronic cardiac decompensated	7	5.64	6.44	7	5.36	38.7		28.6	10.1	43.7
	8	5.45		8	5.33					
	9	5.43		9	5.59					
D. O. C., chronic cardiac decompensated	3	5.29				40.6	42.1	26.7	13.9	38.2
	4	5.71								
	5	5.56								
	6	5.74								
	7	4.89	7.79	7	5.43	36.0		25.3	10.7	
	8	4.92		8	5.15					
	9	5.04		9	5.07					
Averages (normal subjects and compensated cardiacs)						45.0		30.2	5.4	
Averages (decompensated cardiacs)						41.4		32.3	9.1	

patients with cardiac decompensation, however, the oxygen unsaturation of the venous blood is much greater (19) and more variable. The use of a standard correction factor may therefore involve considerable error.

SUMMARY AND CONCLUSIONS

The Haldane method of obtaining alveolar air has been employed in the study of a series of cardiac patients with dyspnea with no greater variation in results than is found in studies of trained normal subjects. The carbon dioxid tension of alveolar air thus obtained has been found consistently low in comparison with the carbon dioxid capacity of venous plasma. The comparatively low values are not due to technical or subjective errors nor to any considerable increase in the volume of the dead space. The patient with cardiac decompensation maintains his alveolar CO_2 at a lower level than does the normal and the level to which he will permit it to rise under the influence of rebreathing is proportionately reduced.

The Plesch method gives variable results according to the number of respirations and the duration of rebreathing. If, however, an average of a series of observations in which these factors have been varied is employed the results agree with those given by the Henderson method for venous alveolar CO_2. The values found by these methods are somewhat lower in decompensated cardiac subjects than in normal persons; but are high in relation to the values given by the Haldane method.

No attempt has been made to translate alveolar CO_2-tension into terms of arterial or venous CO_2-tension. The alveolar air has been considered entirely from a functional standpoint as that portion of the air in the lungs which is available for the exchange of gases between the blood in the pulmonary circulation and the outside air. If the term "alveolar air" is used in this sense, it may be said that the alveolar CO_2-tension of subjects with cardiac dyspnea is low in comparison with the carbon dioxid capacity of the plasma. As the latter is variable, but seldom abnormally high, the alveolar CO_2-tension is usually not only relatively but absolutely diminished.

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STUDIES OF THE RESPIRATORY MECHANISM IN CARDIAC DYSPNEA

II. A Note on the Effective Lung Volume in Cardiac Dyspnea

JOHN P. PETERS, Jr. and DAVID P. BARR

From the Russell Sage Institute of Pathology, in affiliation with the Second Medical Division, Bellevue Hospital, and the Department of Medicine, Cornell University Medical College

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That the vital capacity of the lungs is reduced during cardiac decompensation is a well-established fact (1), (2). A few experiments have been conducted to find out whether this reduction is associated with a change in the total air containing space of the lungs and to ascertain the effect of such a change on the functional efficiency of the respiratory mechanism.

METHODS

The problem has been approached in two distinct ways: *a*, by the application of the Lundsgaard (3) method for measuring the lung volume by rebreathing oxygen; *b*, by a study of the effect of continuous rebreathing of air on the volume of the tidal air and the comparison of the latter with the vital capacity.

The lung volume of a few normal and pathological cases was measured by the Lundsgaard (3) method of rebreathing oxygen. A graphic record of the preliminary respirations and the vital capacity was obtained with a Tissot spirometer by means of a combination of valves similar to that used for rebreathing experiments and described in the preceding paper (4). A rebreathing bag containing a measured amount of oxygen was attached in place of the Haldane tube. In all cases the subject commenced rebreathing from the position of complete expiration. The volume of the residual air was calculated from the gas mixture in the bag at the end of the observation. The volume of the vital capacity was obtained from the graphic record. The values thus obtained were compared with those obtained by the Lundsgaard (3) method of chest measurement.

TABLE 1

Vital capacity and lung volume

NAME AND CONDITION	DATE	VITAL* CAPACITY	RESIDUAL AIR*	LUNG* VOLUME	METHOD OF DETERMINATION			REMARKS
					Volume of O_2 re-breathed	Number of respirations	Time of rebreathing	
		cc.	*cc.*	*cc.*	*cc.*		*seconds*	
1. D. P. B., normal	*Lundsgaard measurements*	*3332**†	*1478**†	*4810**†				Low Lundsgaard measurement probably faulty and due entirely to excessively short sternum
	v/19............	4429	1662	6091†	3000	7	18	
	v/21............	4328	1551	5879†	3000	7	15	Ratio V. C./Surface Area = 2.73
	v/22............	4239	1715	5954†	2050	7	17	
		4284	1632	5916†	2010	7	15	
2. J. P., normal	*Lundsgaard measurements*	*3704*	*1609*	*5313*				
	v/22............	3772	1621	5393	3000	7	17	
		3876	1723	5599	2000	7	17	
3. J. C., chronic cardiac	*Lundsgaard measurements*	*2980*	*1460*	*4390*				Young boy of 18 with early rheumatic endocarditis Compensated at rest
	vi/10/10.........	2345	1560	3905	2070	7	18	No dyspnea nor hyperpnea at rest. Lungs clear
		2432	1515	3947	2070	7	18	

4. J. A., chronic cardiac	*Lundsgaard measurements*	*2930*	*1460*	*4390*				Chronic cardio-nephritic. Hypertension. Cardiac hypertrophy. Blood pressure 210
	v/16/19..........	2430						No dyspnea nor hyperpnea while at rest. Lungs clear
	vi/7/19..........	1242 1329 1418	1214 1250	2456 2579	2020 1500	7 7	19 17	Some hyperpnea even when at rest in bed. Lungs clear except showers of râles at right base
5. A. R., chronic cardiac	*Lundsgaard measurements*	*3640*	*1740*	*5380*				At time of examination was found to have very little hyperpnea. Also had a reduction of the plasma bicarbonate. The hyperpnea was probably due to the reduction of the alkaline reserve and not to cardiac factors. Chest clear. Periodic breathing
	vi/27/19..........	1489 1789	1744	3233	1510	7	17	
6. W. W., chronic cardiac	*Lundsgaard measurements*	*4698*	*2315*	*7013*				Moderate hyperpnea. Some dulness and râles at extreme bases on both sides
	vi/24/19..........	1174	2320	3494	1500	7	14	
	vi/25/19..........	1452	2190	3642	1535	7	20	

TABLE 1—*Concluded*

NAME AND CONDITION	DATE	VITAL* CAPACITY	RESIDUAL AIR*	LUNG* VOLUME	METHOD OF DETERMINATION			REMARKS
					Volume of O_2 rebreathed	Number of respirations	Time of rebreathing	
		cc.	*cc.*	*cc.*	*cc.*		*seconds*	
7. R. W. chronic cardiac	*Lundsgaard measurements*	*3930*	*1870*	*5800*				No dyspnea when at rest. Some impairment of resonance at extreme right base
	vi/23/19..........	1714 1064	1264 1244	2978 2308	2050 2000	7 7	17 17	
	vi/24/19..........	1869						
	vi/30/19..........	2080	1091	3171	2040	7	17	
8. D. O. C., chronic cardiac	*Lundsgaard measurements*	*3540*	*1800*	*5340*				
	iv/29/19..........	1303						Marked dyspnea when at rest. Some signs of fluid at right base
	vi/2/19..........	920 985	757 799	1677 1784	2020 1040	7 15	Very rapid 17	Some fluid in right chest
	vi/6/19..........	1193	873	2066	1060	15	Very rapid	Dyspnea more marked. Fluid to angle of right scapula; 1450 cc. removed just after experiment
	vi/9/19..........	1068 1132	887 861	1955 1993	990 1000	12 15	17 17	Dyspnea less marked. Fluid above angle of right scapula. Some dulness at extreme left base

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* In each case the values for the normal lung volume as determined by the Lundsgaard method of chest measurement are reported first, in italics. Below them appear the values obtained by the rebreathing method.

† The great discrepancy between the observed and the calculated lung volumes of DPB seems to be due to a very short sternal measurement. The relation between his vital capacity and surface area is quite normal, 2.73. In the light of the work of Dryer and that of West it appears that the surface area is a better criterion from which to calculate the vital capacity. Unfortunately we have not sufficient data to permit recalculations on this basis.

The apparatus and method employed in the rebreathing experiments were described in the first paper of this series (4). By a study of the tidal air of normal subjects and cardiac patients after the continuous rebreathing of air from a spirometer, we hoped to gain some information on the effect of the reduction of vital capacity on the functional efficiency of the respiratory mechanism.

RESULTS

The results of the lung volume determinations are collected in the first three columns of table 1. In each case the normal lung volume, as calculated by the Lundsgaard (3) method of chest measurement is reported first, in italics. Below it appear the values obtained from the rebreathing experiments. In the fourth, fifth and sixth columns are given the conditions of the experiments: the volume of oxygen taken in the rebreathing bag; the number of respirations and the duration of the rebreathing.

The first two subjects were normal. The third had chronic cardiac valvular disease without decompensation. His vital capacity, residual air and lung volume were almost normal. Number 4, at the first observation, in the absence of dyspnea, showed only a slight reduction of the vital capacity. Three weeks later, with a recurrence of dyspnea, his vital capacity was found to be only half as large. His residual air was not far from normal. Unfortunately his residual air was not determined at the time of the first observation.

Number 5, A. R., had hypertensive nephritis with some edema and signs of uremia: drowsiness and Cheyne-Stokes breathing. He showed no considerable hyperpnea, although his plasma bicarbonate was reduced. His chest was entirely clear. His vital capacity was very small but his residual air practically normal.

The last three were decompensated patients with dyspnea and showed very low vital capacities. The volume of the residual air was also considerably below normal in the last two. With the exception of the last patient, who had a massive hydrothorax, the physical signs found in the chest were insignificant in comparison with the diminution of lung volume.

These results indicate that, in patients with cardiac dyspnea, the total volume of air in the lungs which can be detected by the usual methods is considerably less than that found in normal persons. The largest part of the reduction in lung volume is due to a diminution of the vital capacity. The residual air is unchanged or slightly reduced.

The results of the rebreathing experiments are given in table 2. The subjects are arranged from above downward according to the magnitude of their vital capacities, which appear in column 1. In column 2 is given the normal vital capacity as calculated from the Lundsgaard (3) chest measurements. In column 3 is shown the average resting tidal air and in column 4 the tidal air at the end of a rebreathing experiment. The last we have called the "maximum tidal air" because it is the largest respiratory volume which the subject will exchange under the influence of the strongest respiratory stimulus that can be applied, carbon dioxid.

TABLE 2

Vital capacity and tidal air

NUMBER AND NAME	VITAL CAPACITY	CALCULATED VITAL CAPACITY LUNDSGAARD	RESTING TIDAL AIR	TIDAL AIR AT END OF REBREATHING	DIAGNOSIS AND REMARKS
1. D. P. B...	4537	3332*	580	2407	Normal
2. J. P.......	3953	3704	372	1929	Normal
3. T. deC....	3839			1249	Cardiac. Compensated
4. P. K......	3503		371	1192	Gastric neurosis
5. A. J......	2729		389	980	Shell shock. Effort syndrome
6. J. B......	2519		737	1236	Cardiac. Slight dyspnea and hyperpnea
7. J. C......	2432	2980	421		Cardiac. Compensated
8. J. A......	2430	2930	439	1116	Cardiac. Compensated
9. C. D......	2145		315	581	Cardiac. Severe decompensation
10. J. M. L...	1789		390	1309	Cardiac. No dyspnea nor hyperpnea
11. A. R......	1789	3640	604		Nephritic. Mild acidosis
12. R. W.....	1714	3930	244		Cardiac. Some dyspnea
13. J. J. F....	1704		304		Cardio-nephritic. Mild acidosis
14. W. W.....	1452	4698	469		Cardiac. Some dyspnea
15. D. O. C...	1303	3540	423	638	Cardiac. Marked dyspnea
16. S. I.......	1248		375		Cardiac. Severe dyspnea
17. J. M......	1197		475		Cardiac. Extreme cyanosis. Some dyspnea
18. Jos. C....	1182		385	492	Cardiac. Marked dyspnea

* See note, table 1.

Peabody (5) found the average resting tidal air slightly reduced in cardiac dyspnea. This our figures do not show. If such a reduction exists it is small in proportion to the enormous reduction found in the vital capacity.

The maximum tidal air, on the other hand, varies almost directly with the vital capacity and the ratio between the two shows the same range of variation in cardiac patients with dyspnea as in normals (0.35 to 0.55). Number 10 is the only exception to this rule. In his case the "maximum tidal air" was very large in proportion to the vital capacity. It was noted in the course of the experiment on this patient that he could not be induced to exert himself to the full extent of his strength when he blew into the spirometer. The low vital capacity, in this instance, may be only an expression of lack of effort on the part of the patient. This was completely overcome under the stimulus of carbon dioxid and he showed a normal "maximum tidal air," although he did not continue rebreathing to the point of exhaustion.

In the case of the decompensated cardiac patients, however, there was no lack of effort apparent during the determination of the vital capacity. Moreover, two of them continued rebreathing to the point of extreme exhaustion. The reduction of the "maximum tidal air" and the vital capacity may be a functional matter, due to reflex inhibition. If so, this inhibition is so profound that it can not be overcome by the strongest stimulus which can be applied. The increase in the tidal air which normally occurs in response to the stimulus of carbon dioxid is limited by the lowered vital capacity.

DISCUSSION

Siebeck (1), in 1912, measured the lung volume in a series of cardiac patients by a rebreathing method. His studies showed that in cardiac dyspnea the residual air was relatively increased, although the absolute values he obtained seem to have been lower than normal. The reserve air and the complementary air were both decreased. These results are substantially the same as ours. However, Siebeck regarded them as questionable because he was unable to obtain the same constancy in his rebreathed mixtures in cardiac patients as in normals. In the latter the concentration of hydrogen in the rebreathing bag became constant after 5 respirations. In subjects with cardiac dyspnea the concentration continued to change even after 10 respirations. This Siebeck considered to be due to improper diffusion of air in the lungs. He concluded that the actual volume of residual air was greater than that indicated by the rebreathing methods.

As Sonne (6) has shown, the residual air can be measured with only an approximate degree of accuracy even in the normal person. Whether Siebeck is right in believing that the amount of air present is greater than that found, we are unwilling to argue. In two or three cases the number of respirations, the time and the volume of air rebreathed were varied without significant changes in the values obtained. This does not agree with Siebeck's findings. But any air that may be present and undetected must be peculiarly useless for respiratory purposes. A functional conception of the lungs is of more value than a purely anatomical one. In a normal person residual air determinations may be considered as measurements of the volume of air space in the fully deflated lungs, which is available for the rapid diffusion of gases. In this sense, certainly, the residual air is not increased in cardiac dyspnea. Since the vital capacity is decreased the effective volume of the lungs must be diminished.

Whether this diminution is due to a true anatomical lesion or not, we are unprepared to say. In some instances hydrothorax plays a part, but it is by no means an essential part. This we may assume not only because of the absence of physical signs of fluid, but also because of the rapidity with which the vital capacity increases as compensation is established (7).

Just what effect such a diminution of the lung volume may have on the gas exchange must be largely a matter of speculation until the cause of the diminution is determined. If it is not due to a true anatomical lesion it is quite possible that some or all of the blood in the pulmonary vessels is not in gaseous equilibrium with the alveolar air. This might, in part at least, explain the discrepancy between the alveolar CO_2 and the CO_2-combining capacity of the plasma.

This discrepancy Pearce (8) has attributed entirely to the effect of stasis in the general circulation. He has drawn a fine distinction between the causes of the low alveolar CO_2-tension in congenital heart disease, pneumonia and acquired heart disease. In congenital heart disease part of the venous blood does not pass through the lungs, and in consequence is given no opportunity to rid itself of the CO_2 which it has received from the tissues. In consequence the arterial blood is a mixture of aërated and unaërated blood. If there were no compensatory reaction a true carbon dioxid acidosis would occur. The respiratory mechanism, however, responds to the stimulus of the carbon dioxid. The alveolar CO_2-tension and the CO_2-tension of the blood which does pass through the pulmonary circulation is reduced sufficiently to restore the CO_2-tension and hydrogen-ion concentration of the arterial blood to normal.

In patients with pneumonia in whom Pearce found the same discrepancy between the alveolar CO_2-tension and the plasma bicarbonates, he advances a similar explanation. In this case, although all the blood passes through the pulmonary circulation, he supposes that part of the blood passes through non-aërated portions of the lungs. On what seems to us insufficient evidence, he denies the possibility of such a condition in acquired cardiac disease. But certainly the reduction of the effective lung volume renders it not only possible, but not improbable. Even if Siebeck is right and this diminution is not due to a decrease in the actual air-containing space in the lungs, a certain part of this air must be unavailable for the rapid diffusion of gases and that portion of blood in the pulmonary circulation which comes in contact with this air must be less effectively ventilated than normal.

That the blood flow is retarded in decompensated cardiac disease is probable in the light of Harrop's (9) observation of an increase in the difference of the oxygen content of arterial and venous blood.

That this is the sole factor in the production of the low alveolar CO_2 remains to be proved. It is doubtful whether the method of Christiansen, Douglas and Haldane (10), which was employed by Pearce for the determination of the rate of blood flow, can be interpreted quantitatively in cardiac dyspnea. In this method it is assumed that the CO_2-tension of the alveolar air is the same as that of the arterial blood. That such a relation obtains in cardiac dyspnea demands definite proof. The use of respiratory methods that are designed to determine the venous CO_2-tension is also open to question when applied to conditions in which the venous oxygen unsaturation is excessive. Christiansen, Douglas and Haldane showed that the effect of oxygen on the carbon dioxid dissociation curve necessitated the introduction of a correction for the oxygen unsaturation even in normal persons. The extent of this correction in cardiacs can only be guessed.

Although the effect of the small effective lung volume on the exchange of gases between the blood and the alveolar air remains largely a matter of speculation, its effect on the mechanics of respiration is fairly clear. Here we have to deal only with the vital capacity, the portion of the lung volume available for ventilation of the lungs.

Apparently the reduction of vital capacity which occurs during cardiac dyspnea has little effect in limiting the respiratory exchange during rest. The volume of the resting tidal air is not diminished. On the other hand, the amount by which the tidal air can increase under the stimulus of carbon dioxid seems to be distinctly limited by the reduction of the vital capacity. The reserve of the mechanical apparatus of respiration is therefore greatly diminished.

CONCLUSIONS

1. In cardiac patients with dyspnea no evidence of an increased residual air was obtained by the Lundsgaard method.

2. As the vital capacity is diminished, this means that the effective lung volume, the volume of air in the lungs available for the exchange of gases, is diminished.

3. The maximum volume of tidal air attained under the stimulus of continuous rebreathing is lower than normal in cardiac dyspnea. The reduction in the maximal tidal air bears a close relation to the reduction in vital capacity.

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STUDIES OF THE RESPIRATORY MECHANISM IN CARDIAC DYSPNEA

III. THE EFFECTIVE VENTILATION IN CARDIAC DYSPNEA

D. P. BARR AND JOHN P. PETERS, JR.

From The Russell Sage Institute of Pathology, in affiliation with the Second Medical Division, Bellevue Hospital, and the Department of Medicine, Cornell University Medical College

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In the previous papers of this series (1), (2) it has been shown that the effective lung volume is decreased during the dyspnea of cardiac decompensation. The alveolar CO_2 tension, as determined by the Haldane method, is usually low and always lower than the concentration of the bicarbonate in the venous plasma would indicate. We have shown that the air obtained by Haldane's method is probably true alveolar or exchange air. In this paper we shall present evidence to substantiate this contention and shall discuss the influence of a low alveolar CO_2 tension and of a diminished lung volume upon the effective ventilation in relation to the dyspnea of heart disease.

Peabody, Wentworth and Barker (3) find that, although the level of metabolism is normal or only moderately increased during cardiac decompensation, the minute volume of respiration is much greater than in normal individuals. The increase is accomplished by a more rapid respiratory rate with a moderate diminution in the volume of each expiration. The percentage of CO_2 in the expired air is diminished.

Peabody recognizes that the greater observed minute volume may represent no increase in the amount of effective air breathed. The tidal air consists of two parts. The dead space, which fills the upper respiratory tract is practically atmospheric air and is of no value in the ventilation of the functioning portions of the lungs. It is only the remainder of an expiration, the exchange air, which is effective for purposes of respiration. In any given expiration, the relative proportion of dead space to exchange air will determine the efficiency of ventilation. If, as Peabody assumes, the volume of the dead space is not changed during decompensation while the volume of tidal air is diminished, the amount of effective air in each expiration will be diminished.

It is possible to arrive at a rough estimate of the effective minute volume by subtracting an average dead space value from the volume of the tidal air and multiplying the remainder by the respiratory rate. Different observers, Haldane and Priestley (4), Krogh and Lindhard (5) and Pearce (6), have found the volume of the dead space in normal resting subjects to vary between 100 and 200 cc. with an average of about 130 cc. Since there is no evidence that the dead space is changed in cardiac dyspnea and experiments reported in a previous paper (1) indicate that it cannot be greatly above the normal values, 130 cc. has been used as the average volume of the dead space for purposes of calculation.

To determine the effective ventilation, we made a considerable number of observations on the minute volume of normal individuals and of compensated and decompensated cardiacs. The method did not differ from that of Peabody except that a nose clip and rubber mouthpiece were substituted for the Siebe Gorman mask. The instrumental dead space was 30 cc. The expiratory air was collected in an accurately balanced Tissot spirometer for periods varying from five to ten minutes, the volume of expiration and the number of respirations being recorded for each minute. Volumes were reduced to standard conditions of 760 mm. Hg. and 0°C. To these observations we applied the following formula:

(Tidal Air − 160[1]) Respiratory Rate = Effective Minute Volume

The results are tabulated in table 1.

For the purpose of comparing our results with those of Peabody we have calculated the effective minute volume from his figures. The dead space of the Siebe Gorman mask which he used has been estimated at about 50 cc. Averages of the results are given in table 2.

The results of the two experiments are in practical agreement. In the decompensated cases, the minute volume of respiration is greater and the respiratory rate is increased. The volume of the tidal air is sometimes slightly diminished but this is by no means invariable as is shown by the large tidal air of J. B. and J. D. B. (table 1). The effective minute volume is much greater during decompensation.

It might be argued that the increase in effective ventilation is due to the higher level of metabolism and the consequent increase in CO_2

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[1] To the average dead space of the individual has been added the instrumental dead space of 30 cc.

production which is observed in many dyspneic cardiac patients. That this is not true can be demonstrated by calculations from Peabody's figures (table 3).

Figures for surface area show that the average size of the patients in the two groups is practically identical. Differences either in the production of CO_2 or in the effective ventilation cannot be due to discrepancies in size. The CO_2 production per minute is only 5.1 per cent greater in the decompensated cases. The volume of effective air breathed per minute is 30.3 per cent higher than in the cases without

TABLE 1

SUBJECT	MINUTE VOLUME	TIDAL AIR	RESPIRATIONS PER MINUTE	EFFECTIVE MINUTE VOLUME	DIAGNOSIS AND REMARKS
	cc.	cc.			
D. P. B.	6,900	532	13.0	4,822	Normal adult
	6,789	580	11.7	4,913	
	6,040	592	10.2	4,406	
	6,751	668	10.1	5,131	
J. P.	5,524	372	14.9	3,148	Normal adult
	4,898	389	12.6	2,885	
	7,127	520	13.7	4,943	
G. C. D.	6,386	484	13.2	4,277	Normal adult
	6,243	488	12.8	4,198	
P. K.	4,659	293	15.9	2,115	Gastric neurosis. No sign of cardiac or pulmonary disease
	7,764	371	20.9	4,420	
Cap.	7,866	333	23.6	4,083	Normal adult. Respirations rapid and variable. Obviously over-ventilating
J. A.	6,474	439	14.8	4,106	Chronic cardiac valvular disease. No dyspnea while at rest in bed
	7,611	408	18.6	4,626	
J. M. L.	4,553	390	11.6	2,675	Chronic cardiac valvular disease. No dyspnea while at rest in bed
	6,133	438	14.0	3,893	
R. W.	5,826	244	23.9	2,003	Chronic cardiac valvular disease. Breathing quietly while at rest in bed. Rapid and superficial during experiment
J. W.	7,680	410	18.7	4,675	Chronic nephritis. Hypertension. No dyspnea while at rest in bed
Average	6,401	442	15.2	3,973	

TABLE 1—*Concluded*

SUBJECT	MINUTE VOLUME	TIDAL AIR	RESPIRATIONS PER MINUTE	EFFECTIVE MINUTE VOLUME	DIAGNOSIS AND REMARKS
	cc.	*cc.*			
J. B.	13,170	737	17.9	10,310	Chronic cardiac valvular disease. Dyspnea and cyanosis while at rest
	10,210	612	16.7	7,540	
Jos. C.	13,590	385	35.3	7,936	Chronic nephritis with hypertension. Severe cardiac decompensation with marked dyspnea
	14,140	350	40.3	7,638	
C. D.	9,982	315	31.8	4,916	Chronic cardiac valvular disease. Severe dyspnea and orthopnea
S. I.	6,930	375	18.5	3,970	Chronic cardiac valvular disease. Massive hydrothorax. Dyspnea and orthopnea
D. O. C.	8,069	423	19.1	5,020	Chronic cardiac valvular disease. Marked cyanosis and moderate dyspnea while at rest. Right hydrothorax
W. W.	9,528	469	20.3	6,279	Chronic cardiac valvular disease. Moderate dyspnea while at rest
J. R.	11,393	311	36.7	5,542	Chronic cardiac valvular disease. Marked dyspnea and orthopnea while at rest
	10,110	357	28.3	5,575	
P. O. S.	9,087	279	32.6	3,879	Chronic cardiac valvular disease. Right hydrothorax. Marked dyspnea and orthopnea while at rest
J. D. B.	14,310	821	17.4	11,501	Chronic cardiac valvular disease. Marked dyspnea and orthopnea while at rest
	12,800	538	23.8	8,996	
J. M.	8,652	475	18.2	5,733	Chronic cardiac valvular disease. Extreme cyanosis and some dyspnea while at rest
	9,843	496	19.8	6,653	
Average	10,788	463	25.1	6,766	

dyspnea. Only a small part of the increase in effective ventilation can be accounted for by a greater CO_2 production.

Furthermore, since the dyspneic cardiac breathes a much greater amount of CO_2, each volume of his effective or alveolar air must contain a smaller percentage of CO_2.

TABLE 2

	COMPENSATED CARDIACS	DECOMPENSATED CARDIACS
Minute volume (cc.*)	5,901	8,521
Tidal air (cc.)	466	398
Respiratory rate	13.2	21.8
Effective minute volume (cc.)	3,530	4,600

* Minute volumes have been recalculated from the CO_2 produced per minute and the percentage of CO_2 in the expired air.

TABLE 3

	COMPENSATED CARDIACS	DECOMPENSATED CARDIACS
Surface area (square meters)	1.72	1.77
CO_2 production per minute (cc.)	196	206
Effective minute volume (cc.)	3,530	4,600

It is possible to show more directly the probability of a reduced alveolar CO_2 tension. In the paper describing their method of obtaining alveolar air, Haldane and Priestley (4) indicated a means of determining the volume of the dead space. The data necessary for the calculation were the volume of an expiration, the percentage of CO_2 in the expired air and the percentage of CO_2 in the alveolar air. These were combined in the following formula:

Formula I

$$\text{Tidal air} - \frac{\text{Tidal air} \times \text{per cent } CO_2 \text{ in expired air}}{\text{Per cent } CO_2 \text{ in alveolar air}} = \text{Dead space}$$

By a simple inversion of their formula, the percentage of CO_2 in the alveolar air can be deduced.

Formula II

$$\frac{\text{Tidal air} \times \text{per cent } CO_2 \text{ in expired air}}{\text{Tidal air} - \text{Dead space}} = \text{Per cent } CO_2 \text{ in alveolar air}$$

Both of these formulae were applied to observations on a number of normal individuals and on cardiacs in varying degrees of decompensation. Minute volume determinations were made as in the previous experiments. At the close of an observation, the air was thoroughly mixed in the spirometer and a sample was taken for analysis. Haldane

TABLE 4

SUBJECT	MINUTE VOLUME	RESPIRATIONS PER MINUTE	TIDAL AIR	CO_2 IN EXPIRED AIR	ALVEOLAR CO_2 CALCULATED FROM DEAD SPACE FORMULA	ALVEOLAR CO_2 OBSERVED HALDANE METHOD	ALVEOLAR CO_2 CALCULATED FROM PLASMA BICARBONATE	DEAD SPACE CALCULATED FROM		DIAGNOSIS AND REMARKS
								Observed alveolar CO_2	Alveolar CO_2 calculated from plasma	
	cc.		cc.	per cent	per cent	per cent	per cent	cc.	cc.	
D. P. B.	6,040	10.2	592	3.92	5.39	5.39		130		Normal adult
	6,751	10.1	668	4.16	5.47	5.40		123		
J. P.	4,898	12.6	389	3.84	6.52	5.89		105		Normal adult
	7,127	13.7	520	4.29	6.14	5.75		132		
Cap.	7,866	23.6	333	3.27	6.29	6.31		130		Normal adult
G. C. D.	6,386	13.2	484	3.42	5.11	6.39		195		Normal adult
	6,248	12.8	488	4.04	6.01	6.63		161		
J. W.	7,680	18.7	410	3.03	4.98	4.85	5.60	124	158	Chronic cardiac valvular disease. No dyspnea while at rest
Average	6,625	14.4	486	3.75	5.74	5.83		138		
J. R.	11,396	36.7	311	2.16	4.44	4.60	6.73	139	181	Chronic cardiac valvular disease. Dyspnea and orthopnea while at rest
	10,110	28.3	357	2.33	4.26	3.95		115		
J. D. B.	14,310	17.4	821	2.14	2.66	3.11	5.53	226	473	Chronic cardiac valvular disease. Moderate dyspnea and cyanosis
	12,800	23.8	538	2.69	3.82	4.53		189		
J. M.	8,652	18.2	475	2.40	3.62	3.39	5.76	109	247	Chronic cardiac valvular disease. Extreme cyanosis, moderate dyspnea
	9,843	19.8	496	2.72	4.02	4.96	7.41	195	284	
P. O. S.	9,087	32.6	279	2.30	5.39	5.69	7.40	136	162	Chronic cardiac valvular disease. Severe decompensation with dyspnea and orthopnea
Average	10,885	25.2	460	2.30	4.03	4.32		150		

samples of alveolar air were taken before and after the minute volume determinations. A specimen of blood was drawn from an arm vein for determination of the bicarbonates in the plasma. The results obtained from the plasma were converted into terms of percentage of alveolar CO_2 according to the method of Van Slyke (7).

In formula II, average dead space values were employed for the calculation of the probable percentage of CO_2 in alveolar air. The results are recorded in table 4 (column 6). They agree closely with the results of direct observation by the Haldane method (column 7) and consequently in the decompensated cardiac lie far below the value indicated by the plasma (column 8).

In formula I, the observed alveolar CO_2 per cent and the percentage indicated by the plasma have been applied to calculate the probable dead space. Substitution of the observed values give volumes of dead space which are within the range of normal (column 9) while substitution of percentages indicated by the plasma give decidedly improbable results (column 10).

Formula II has also been applied to Peabody's figures. Averages are given in table 5.

TABLE 5

	COMPENSATED CARDIACS	DECOMPENSATED CARDIACS
Tidal air (cc.)	466	398
CO_2 in expired air (per cent)	3.35	2.44
Calculated alveolar CO_2 per cent (using dead space of 180 cc.)*	5.72	4.70

* Average dead space of 130 cc. to which is added the extra dead space of 50 cc., the capacity of the Siebe Gorman mask.

Both in Peabody's figures and in ours, the percentage of CO_2 in the alveolar air is lower during decompensation.

DISCUSSION

In this series of papers the question of the concentration of CO_2 in alveolar air during cardiac decompensation has been approached from several angles. Of the direct methods, that of Haldane which has been found applicable to dyspneic patients gives values for alveolar CO_2 percentage much lower than it does in normal individuals. Duplicate samples, however, show quite as close agreement in dyspneic cardiacs as in trained normal subjects. Experiments have shown that the low content is not due to subjective errors nor to an increased dead space. After breathing increasing percentages of CO_2, the alveolar CO_2 percentage of dyspneic cardiacs is still relatively lower than it is in normal individuals under the same circumstances. The percentage, moreover, is not increased by increasing the depth of expiration. An expiration of maximum depth contains no greater concentration of CO_2 than does one of a volume just sufficient to clear the dead space. Whether the specimen of air obtained by the Haldane method is from the alveoli or from other portions of the lungs is not of great importance in the present discussion. Whatever the anatomical source of the sample may be, it is the only air which by the greatest effort the decompensated cardiac can expire. It is the only air which can be functionally effective in the elimination of CO_2 from the body. If this is true the CO_2 contained in this air should completely account for the total CO_2 elimination. In this paper it has been shown by the use of Haldane's dead space formula that the percentage of CO_2 found in the Haldane specimen is the percentage theoretically required to account for the CO_2 eliminated.

The accumulated evidence, both direct and circumstantial, indicates that the percentage of CO_2 is low in the effective air of dyspneic cardiac subjects. The cause of this important phenomenon is not clearly understood. Its chief importance in the present discussion lies in its effect upon the ventilation in cardiac disease.

A low percentage of CO_2 in the effective air necessitates a larger effective ventilation to accomplish CO_2 elimination. Even when the metabolism is normal, the decompensated cardiac exhibits hyperpnea. As long as he lies quietly in bed, this usually involves an increase in the rate of respiration with little or no change in the volume of each respiration. Under these circumstances the low effective lung volume, which is the constant accompaniment of decompensation, is not a factor of great importance in the causation of dyspnea. It probably exerts a greater influence during conditions involving a greater production of CO_2.

Concerning the mechanism of ventilation during exercise, we have accumulated no direct evidence. A good indication of the response is furnished, however, by experiments upon the rebreathing of CO_2. Under these circumstances the cardiac is subjected to a most powerful stimulus to respiration. The mechanism should, by analogy, be similar to that occurring during exercise or any other condition involving a rapid production of CO_2 within the body. It has been shown that the cardiac maintains a relatively low alveolar CO_2 percentage during rebreathing. To accomplish this he must breathe a larger volume of effective air. Under the stimulus of CO_2, the volume of the tidal air increases both in cardiacs and in normal individuals. The increase, however, is in proportion to the vital capacity. In both groups the tidal air reaches a maximum at one-third to one-half of the volume of the vital capacity. Thus, a normal individual with an original vital capacity of 4000 cc. may show under the stimulus of CO_2 a tidal air of 1500 to 2000 cc. In a decompensated cardiac whose vital capacity may be only 1500 cc. the tidal air will not rise above 500 to 750 cc. with maximum CO_2 stimulation. The volume of respiration is strictly limited. Any attempt to increase it is accompanied by marked subjective dyspnea.

Two factors in cardiac disease help to explain the dyspnea which is its most constant subjective symptom. The low percentage of CO_2 in the effective air makes an increase in ventilation essential. The diminished effective lung volume makes any large increase difficult or impossible. The first is active under all conditions while the cardiac is decompensated. The second exerts its chief influence when the production of CO_2 in the body is increased.

CONCLUSIONS

1. Air obtained from decompensated cardiacs by the Haldane alveolar method is true exchange air. It corresponds to the alveolar air obtained by the same method in normal resting subjects. It is the only air effective for the elimination of CO_2.
2. During cardiac dyspnea, the bicarbonate content of the plasma gives no indication of the percentage of CO_2 in the exchange air.
3. The minute volume of effective or exchange air is increased during cardiac decompensation.
4. This is not explained by the higher level of metabolism.
5. The greater effective ventilation is necessitated by the low concentration of CO_2 in the exchange air.
6. Great increases in ventilation are impossible because of the diminished effective lung volume of decompensated cardiacs.

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STUDIES ON THE BRAIN STEM

IV. On the Relation of the Cerebral Hemispheres and Thalamus to Arterial Blood Pressure

F. T. ROGERS

From the Hull Physiological Laboratory, University of Chicago

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In a previous report (1) attention has been directed to the poikilothermous condition that follows the removal of the cerebral hemispheres and thalamus in birds and mammals. In an attempt to discover the causes of this condition it was suggested that possibly a general fall of arterial blood pressure with resulting cutaneous dilatation of the blood vessels might be an essential factor. To test this point a method was devised for measuring the arterial pressure in the pigeon and studies of the blood pressure made before and after reduction to the cold-blooded condition. These tests did not give the anticipated results but led to the discovery that the removal of the cerebral hemispheres, which alone does not reduce the bird to the cold-blooded condition, leads to a permanent slight fall in arterial tension. The plan of the work was therefore extended to a study of the arterial pressure in the normal pigeon, the normal variations, effects of various procedures on the normal pressure, and then, the effect of various types of brain lesions on the arterial pressure.

Asher (2) in his review of the vasomotor mechanism, in 1902 wrote: "There are no known facts which indicate the presence of vasomotor centers in the parts of the brain above the medulla oblongata. The higher cerebral centers influence the vasomotor tone exclusively in a reflex manner through the medullary vasomotor center." This interpretation is based on the original experiments of Ludwig's students, Dittmar and Owsjannikow. These classic experiments in 1872 and 1873 demonstrated the localization of the vasoconstrictor centers in the floor of the fourth ventricle.

Since then innumerable studies have demonstrated that reflex influences from the cerebral hemispheres above and the spinal nerves below may play on this center. (Literature cited by Sachs.) It has become common knowledge that loss of the cerebral hemispheres does not necessarily disturb the arterial pressure in acute experiments. It therefore came somewhat as a surprise to find that in a series of decerebrate birds, kept for several weeks or months after removal of the cerebrum, the arterial pressure was uniformly lower than in normal birds.

Porter showed in 1907 that in curarized rabbits and cats removal of the cerebral hemispheres in acute experiments leads to a profound fall in the blood pressure without depression of the reflex excitability of the vasomotor center.

If a blood pressure tracing be made on a dog before and after decerebration by Sherrington's method, it is frequently found that the pressure is somewhat less after decerebration than before. The complications however of the muscular rigidity that follows, render difficult the interpretation of the effects of the operation on the vasomotor center.

All these experiments are acute traumatic experiments subject to all the uncertainties of shock effects. In the birds many of these difficulties can be obviated, for they can be kept alive easily, for an indefinite time after the operation, and spastic paralytic phenomena are wholly lacking provided the hemispheres only are removed. In the pigeon therefore we have a warm-blooded animal which easily withstands the shock of cerebral ablation, lives indefinitely thereafter, and in which the permanent effects on the blood pressure can be studied.

There was the further inducement that the pigeon in comparison with the mammals combines a relatively low type of cerebral development with the warm-blooded condition and it was hoped that some light would be thrown on the nature of the nervous mechanism of heat regulation in its earlier development.

Methods. The measurements of arterial pressure were at first made in the ordinary way of cannula and mercury manometer. It was soon found that a more convenient and just as reliable method was furnished by substituting a hypodermic needle for the cannula and this method was used in all the work here reported. All measurements have been made on the brachial artery using a hypodermic needle, size 19 or 20 with a bore of about 1 mm. diameter. This furnishes a tube which when inserted into the artery slightly stretches it and fits tightly enough to allow no escape of blood. (In very large birds it may be necessary to use a larger needle.) This was connected to an ordinary small size mercury manometer made of glass tubing 4 mm. in diameter, with stiff-walled rubber tubing. In order to avoid any errors due to possible mechanical effects of friction or differences in level between the bird and the level of the mercury, the manometer was set in a fixed position with the level of the mercury in the plane of the tip of the breast muscles when the bird was fastened on its back. The same manometer, two needles of the same size, and the same size and length of rubber connections, were used throughout the series. Hence any mechanical errors will be nearly uniform throughout the comparative series of pressure determinations. Clotting of the blood was prevented by using 7 per cent sodium citrate throughout the apparatus. A lesser concentration was not always satisfactory, but with citrate solution of this concentration the tracing can be continued indefinitely through all variations of blood pressure of zero to 200 mm. When the needle was removed from the artery the blood vessel was doubly ligated. This causes no apparent trouble to the bird, so the collateral circulation must be extensive. In only one case did gangrene follow the ligation and in this case the ligature involved both brachial artery and vein. All readings were made with the birds under ether anesthesia; no other drugs were used in this study. This introduced, of course, the effects of the anesthetic added to that of the cerebral lesion. No other method however seemed available for it is important that there be no struggles by the animal as these will promptly change the level of the arterial pressure. In order to check these variations due to the action of the anesthetic the routine procedure was adopted of etherizing the bird, putting it on its back with wings spread and the needle inserted in the artery. The pressure was then raised in the manometer to a value a little less than the anticipated pressure. Some care was necessary here not to exceed the arterial pressure and thereby kill the bird by forcing citrate into the circulation. This happened twice in the series of experiments. The arterial pressure was then recorded for all depths of anesthesia, varying from light to deep, using rigidity as an index of light, and abolition of the corneal reflex as index of deep anesthesia. The readings of pressure given in the tables are, therefore, those of the extreme variations under anesthesia, but not including variations due to struggles of the animal. In many cases the pressure maintained a nearly constant level and only one figure is given in such cases.

In order to determine whether or not there were errors due to possible differences in size of the arteries of the two wings, readings were made on a series of birds comparing specifically the pressures in each of the two brachial arteries, in the same bird, allowing intervals of several days between determinations, during which time the birds were kept confined in the cages used throughout the series of experiments. These readings (table 5) agree closely. Of course in the case of specific anatomical anomalies such a comparison would be of no value. One such case has been seen, namely, a double instead of a single brachial artery. In a series of determinations however this factor is neutralized in the averages given, and by the consideration that the arteries of both wings were used indiscriminately throughout the series of determinations.

TABLE 1

Arterial pressure in normal pigeons

Anesthesia variations

NUMBER OF PIGEON	ARTERIAL PRESSURE	NUMBER OF PIGEON	ARTERIAL PRESSURE
	mm.		*mm.*
4	122–142	190	104–114
	114–118	188	94–120
	116–118	158	110–130
	150–160	156	154–176
	122–132	178	92–140
153	108–142	161	78–130
170	136	163	108–116
172	103–118	162	106–108
171	135	179	122–160
	116	169	100–110
177	96–108	193	96–98
181	92–108	193	96–102
	126–152	194	122–140
185	116	194	106–124
164	134–176	195	110–148
182	92–118	195	104–128
184	88–128	159	150–170
183	86–112		104–120
186	102–118	168	122–144
187	90–110		

Average pressure, 118 mm.

Average limits of variations, 109–130 mm.

The figure 109 is the average of all the lower of the two readings given for the majority of the animals. Similarly 130 is the average of the higher reading given. The figure 118 is the average of these extremes combined with the single figure given for several birds.

Normal blood pressure. The average arterial blood pressure in the brachial artery under ether anesthesia of thirty-nine normal adult pigeons was found to be 118 mm. (table 1). The extreme limits for all stages of anesthesia were 78 to 176 mm. The average limits for the series are 109 to 130 mm. (fig. 1).

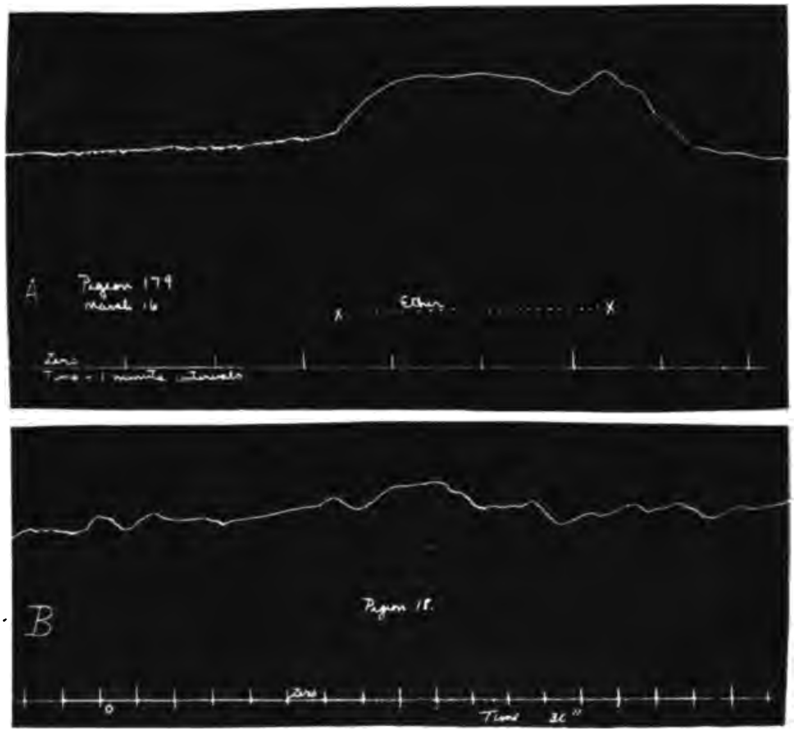

Fig. 1. Arterial pressure in normal pigeons, ether anesthesia. *A:* Light anesthesia: x–x more ether given so as to put bird in a state of deep anesthesia. Time in one minute intervals. *B:* Traube-Hering waves in pigeon under light anesthesia. Time in 30 second intervals.

Note: All figures of blood pressure are reduced one-half, except figure 3, which is reduced one-third. A true scale for measuring pressure in these tracings is given in figure 4.

Mechanical stimulation of the brachial nerves, ammonia to the nostrils, and asphyxia, produced the usual types of vasomotor responses. The effects of these procedures are given in table 2 and figures 2 and 3. Electric stimulation of these nerves was not employed. Mechanical stimulation of the nerves by pinching with forceps or traction caused brisk changes in arterial pressure, both pressor and depressor effects.

A lowering of blood pressure by cardiac inhibition seemed particularly easily elicited by traction of the brachial nerves. Traube-Hering waves of pressure have been frequently observed as also have been the shorter respiratory waves (fig. 1, B).

TABLE 2

Variations of arterial pressure in normal pigeons

NUMBER OF PIGEON	AVERAGE NORMAL PRESSURE	RANGE OF VARIATIONS OF PRESSURE INDUCED BY		
		Mechanical stimulation brachial nerves*	Ammonia to nostrils (rise in pressure)	Asphyxia (increased pressure)
	mm.	*mm.*	*mm.*	*mm.*
	110	14		24
	130		34	
	110		40	
153	125	30		
158	120	50	16	24
169	105	22	22	
178	116	12		
179	140	8	26	22
156	164	16	16	
170	110		32	

* This includes both pressor and depressor effects.

TABLE 3

Effects of slight hemorrhage on arterial pressure

NUMBER OF PIGEON	AMOUNT OF BLOOD LOST	ARTERIAL PRESSURE	
		Before bleeding	10 minutes after bleeding
	cc.		
159	5	150–170	126–128
173	1	110	102–118
155	2	130–150	134–148

The removal of small amounts of blood leads to a fall followed by quick recovery (table 3). Thus the loss of 1 to 2 cc. of blood in the normal pigeon leads to a fall quickly followed by compensatory changes which bring the pressure back to normal. The loss of 5 cc. of blood causes much more profound effects but in ten minutes the blood pressure has again reached the average level although below the previous level in the same bird (pigeon 159, table 3).

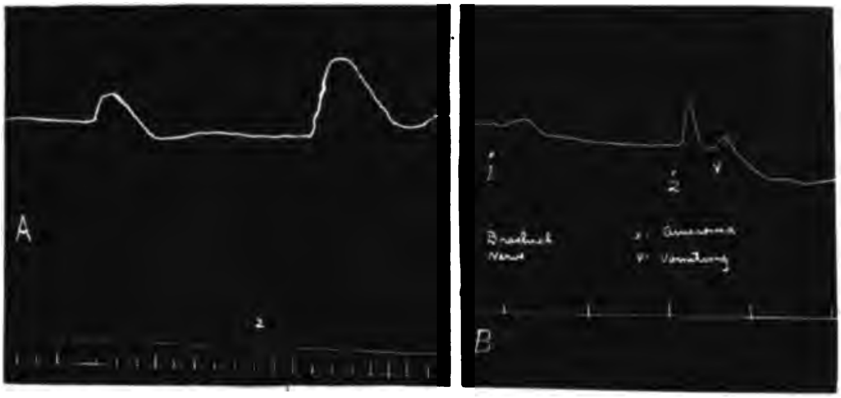

Fig. 2. Blood pressure, normal pigeons. *A: 1*, mechanical stimulation of brachial nerves; *2*, ammonia vapors to nostrils. *B:* Deeper anesthesia than in *A*. *1*, mechanical stimulation of brachial nerves; *2*, ammonia to nostrils; *V*, vomiting.

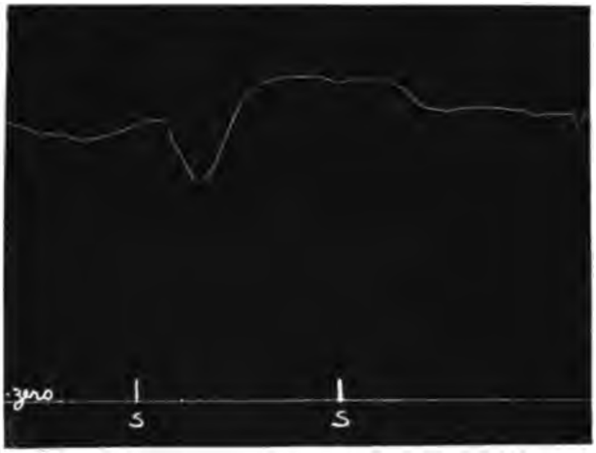

Fig. 3. Blood pressure, normal pigeon. Depressor effects following mechanical stimulation of brachial nerves.

The effect on blood pressure of deprivation of food—but not water—was tested on four birds. These birds were starved for four to eight days. At the end of that time the arterial pressure was normal in three of the birds and slightly lowered in one of them (table 4). The effects of confinement were tested by comparing the pressures of three birds which had been in the laboratory cages for over a year. The average pressure of these birds (six determinations, table 5) was 115 mm. compared with the average pressure of 118 mm.

TABLE 4

Influence of starvation on arterial pressure. Cerebrum intact

NUMBER OF PIGEON	STARVATION PERIOD	ARTERIAL PRESSURE	
		Before starvation	After starvation
	days	*mm.*	*mm.*
183	7	88–112	80–86
184	7	104–114	120–122
182	8	90–108	95–98
164	4	116–135	120–126

TABLE 5

Comparative pressure determinations in the two brachial arteries of the same birds (birds have been in laboratory cages for one year)

NUMBER OF PIGEON	TIME BETWEEN MEASUREMENT	RIGHT WING	LEFT WING
	days	*mm.*	*mm.*
193	3	96–98	96–103
194	3	122–140	106–124
195	3	104–128	110–148

Average pressure in these birds, 115 mm.

By this series of determinations of arterial pressure on thirty-nine normal adult pigeons the average pressure has been determined, the normal variations noted, and the effects of such factors as anesthesia, starvation and the routine reflexes observed. Preliminary to the production of cerebral lesions it was important to note whether or not the loss of the small amount of blood necessary to the operation would alter the level of the arterial pressure. As noted in table 3, the loss of 2 cc. of blood is so quickly compensated for as to justify the conclusion that if in operating not more than 2 cc. is lost the normal level of arterial pressure is maintained.

Effects of decerebration. A description of the varying conditions that follow the removal of the hemispheres or hemispheres and thalamus, or partial lesions of both, has been given elsewhere (7). It will suffice here to state that the classic picture of decerebrate behavior in the pigeon is obtained only if the thalamus be not traumatised in the process of decerebrating (8), (9). Thalamic injury is associated with temperature disturbances, with the behavior of the animal varying as its body temperature changes. The technique of decerebration is therefore important in a comparative series of studies on the rôle of these two parts of the brain. The general anatomy of this region of the brain is described in a previous paper (1). In decerebrating the upper part of the skull above the cerebral hemispheres was removed taking care not to puncture the dura mater. After a little practice this is easily done. A bridge of bone over the very small longitudinal sinus was left intact so as to diminish bleeding and to serve as a support for the skin after removal of the brain substance. The dura was then cut longitudinally and reflected, the anterior cerebral arteries were cauterized and the hemispheres removed in toto. In this way the operation can be completed with the loss of less than 1 cc. of blood. If more than 2 cc. of blood was lost in operating it is indicated in the history of the animals. If the thalamus was to be destroyed, this was done with an electro-cautery, after removal of the hemispheres. The autopsy findings in the brain with histologic description of the parts of the brain remaining after recovery from this type of injury have been described in the preceding report to which reference has been made (7).

It is common knowledge that the removal of the hemispheres sometimes leads to a slight immediate fall in blood pressure which has been attributed to shock, hemorrhage, mechanical stimulation, etc. The same effect is true of the pigeon. In order to avoid these acute effects, the birds have been kept for time intervals of one week to four months after decerebration before the arterial pressure was measured (table 6 and fig. 4). These studies have been carried out on fifteen pigeons, allowing time intervals for recovery from any acute shock effects. In ten of these birds the arterial pressure was measured before decerebration; in five this was not done. For the latter animals some indication of the change in pressure may be gathered by comparing with the average normal arterial pressure value given above.

The average pressure after decerebration, thalamus being left intact, allowing three to seventy-five days for recovery, in fifteen pigeons, was found to be 99 mm., in comparison with the average pressure in thirty-nine normal pigeons, of 118 mm. This reduction seems to be uniform and constant, and continues for months after loss of the hemispheres. It is not due to loss of blood at the time of decerebration for the amount of blood lost was so slight that compensation quickly occurs in the normal animal. It is not due to failure of compensation in the decerebrated bird, for when pressure tracings were made allowing the blood to force the mercury from the zero level, it rises to the level of arterial pressure as quickly in the operated birds as in the normal ones. (Compare figs. 5 and 8 A.) With decerebration plus thalamic lesions, slower compensation may be a factor (see below). This fall in pressure is not due to mere disturbances of food supply for, as tested in normal

TABLE 6

Effects on arterial pressure of removing the cerebral hemispheres, thalamus not traumatised

NUMBER OF PIGEON	TIME FOR RECOVERY*	ARTERIAL PRESSURE		REMARKS
		Before decerebration	After decerebration	
	days	*mm.*	*mm.*	
179	5	120	102	Much bleeding at time of operation
178	5	120–144	120	Good condition
156	5	154–176	96–112	Good condition
158	8	108–128	96–110	Good condition
168	16	118–124	92–120	Good condition
106	75		96–108	Good condition
155	3	126–136	96–98	Good condition
166	16	120–122	104–114	Good condition
167	5	116–124	96–102	Good condition
152	16	82–98	90–95	Good condition
162	3	104–108	88–94	Good condition
157	5		80–84	Good condition
116	11		86–90	Good condition
	21		96	Good condition
	6		87	Good condition

* The words "time for recovery" indicate the time interval elapsing between the removal of the cerebral hemispheres and the final blood pressure determinations. The words "good condition" indicate that the bird exhibited no skeletal muscle incoördination, body temperature was normal, feathers fluffed in the characteristic decerebrate manner and the bird exhibited decerebrate restlessness. Any variation from these characteristic effects usually indicate thalamic or medullary disturbances or incomplete decerebration.

birds (table 4), complete starvation for periods of three to eight days did not cause greater variations in pressure than the normal anesthesia variations. It might be due to one or more of the following factors.

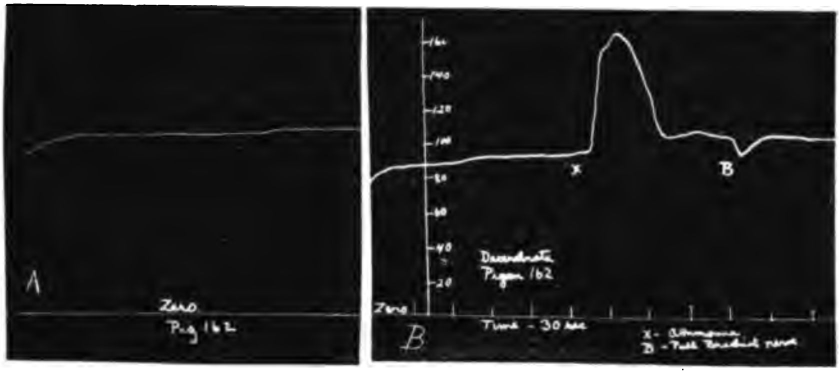

Fig. 4. Blood pressure, pigeon 162: *A:* before, and *B:* eight days after removal of the cerebral hemispheres. Thalamus intact and body temperature normal. Time in 30 second intervals. *B:* *x*, ammonia to nostrils; *b*, mechanical stimulation of brachial nerves.

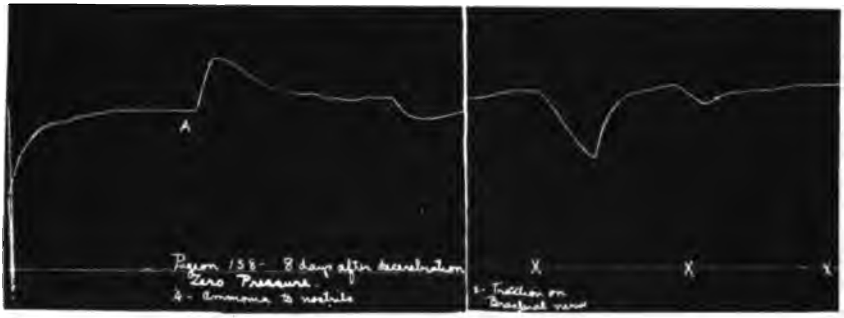

Fig. 5. Blood pressure, pigeon 158, eight days after decerebration. *A:* ammonia to nostrils; *X:* mechanical stimulation of brachial nerves, repeated three times. There was no preliminary increase in pressure in manometer before making this tracing, so the mercury was forced up from the zero line wholly by the arterial pressure.

First, metabolic depression following loss of the hemispheres associated with decreased activity of the animal; or second, loss of a tonic activity of the hemispheres on the vasoconstrictor centers; or possibly due to depressed skeletal muscle tone with resulting capillary dilatation.

In order to test the first of these possibilities the blood pressure determinations were made on a normal pigeon before and after blinding and starvation. Blinding the bird by excision of the eyes brings about a condition of quiet and lessened muscular activity that simulates that of decerebration. The combination of starvation for four days associated with this inactivity of blindness did not lower the average arterial pressure (see table 4, pigeon 164). Of course prolonged starvation for a long period of time will alter the pressure, witness pigeon 183, table 4, and pigeon 188, table 9. Inasmuch as all decerebrate birds were fed by hand and kept in as good condition as possible, it seemed that one week of absolute starvation would represent as great a metabolic disturbance as might be induced by loss of the hemispheres. The result of this test therefore suggested that the fall in arterial pressure is due to the loss of reflex or tonic influences from the cerebrum on some part of the blood pressure regulating mechanism.

In the decerebrate pigeon, thalamus not traumatised, the usual types of vasomotor reflexes may be elicited by stimulation of spinal nerves, ammonia, etc. (fig. 5). Asphyxia produces the usual rise in pressure.

Decerebration with destruction of the thalamus. Combined decerebration and thalamic cauterization abolishes the ability to maintain and regulate the normal body temperature of the pigeon (39° to 41°C.). As stated in the introduction, it was thought that possibly this condition might be due to a generalized fall in blood pressure. As is evident from table 8, this is not the case. If the body temperature of such a bird is kept near the normal level by keeping the bird in an incubator at 30°C. the arterial pressure of such a bird is very near the pressure of the warm-blooded decerebrate pigeon. Thus the average values of blood pressure, decerebrate pigeons (body temperature normal) and decerebrate-thalamic destruction (cold-blooded animals) are as follows:

TABLE 7

BIRDS	NUMBER OF BIRDS	BODY TEMPERATURE	ARTERIAL PRESSURE
		°C.	mm.
Normal	39	39–41	118
Decerebrate	14	39–41	99
Decerebration and thalamus destroyed	7	34–41	99
Decerebration and thalamus destroyed	7	26–33	87

It is conclusively evident from this table that the loss of temperature regulation is not due primarily to a lowered arterial pressure, but that the changes of arterial pressure are secondary to the body temperature changes. As the body temperature rises or falls the blood pressure does likewise (table 8 and fig. 6). To this rule one exception was found, pigeon 174. In this animal the pressure did not fall as the body temperature fell, but acted in an inverse manner. Attempts were made to duplicate this but were unsuccessful. This was a bird in which the operation of decerebration was associated with much bleeding. The writer is inclined to attribute the high arterial pressure in this bird to a possible intracranial pressure complication or incomplete destruction of the thalamus.

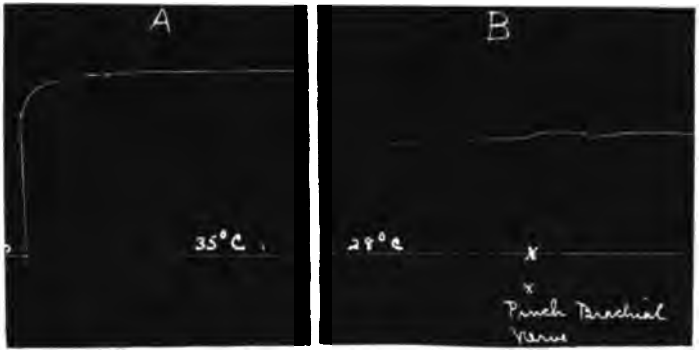

Fig. 6. Blood pressure in pigeon 191. Poikilothermous bird; decerebrate and thalamus destroyed. *A:* Body temperature of pigeon, 35°C. *B:* Body temperature of pigeon, 28°C. *x*, mechanical stimulation of brachial nerves.

In the poikilothermous pigeon the vasomotor reflexes vary in intensity as the body temperature varies (figs. 6 and 7). Both pressor and depressor effects were obtained by stimulation of the brachial nerves. As the body temperature falls the pressor reflexes particularly decrease in intensity. The depressor effects seemed to be due to cardiac inhibition and were present at low body temperatures at which the pressor effects were very much reduced (compare figs. 6 and 7). Ammonia to the nostrils caused a rise in pressure which is associated with respiratory distress. This effect was present at all body temperatures tested (28° to 41°C.), but was more sluggish at lower temperatures than at normal body temperature (compare figs. 5, 7 and 8).

TABLE 8

Arterial pressure in pigeons rendered poikilothermous by destruction of cerebrum and thalamus

NUMBER OF PIGEON	DATE	PROCEEDING	TIME FOR RECOVERY	BODY TEMPERATURE	ARTERIAL PRESSURE
			days	*°C.*	*mm.*
191	June 22	Operation		39	
	June 28	Blood pressure	6	35	93
	June 28	Blood pressure	6	28	58
175	March 9	Operation		39	
	March 14	Blood pressure			
		1 p.m.	5	36	102–106
		6 p.m.	5	28	83–86
174	March 5	Operation, much bleeding		40	
	March 10	Blood pressure			
		2 p.m.	5	34	121
		4 p.m.	5	31	124
		6 p.m.	5	29	124–134
114	February 7	Operation		39	
	February 22	Blood pressure	15	29	92
	March 8	Blood pressure	29	41	85
	March 13	Bird dead			
113	February 7	Operation		3	
	February 20	Blood pressure	13	28	92
		Operation	3	29	78
		Operation	29	41	85
176	March 9	Operation		39	
	March 12	Blood pressure	3	29	76
	March 13	Dead			
180	March 18	Operation, much bleeding		39	
	March 29	Blood pressure	11	34	102–106
169	March 1	Blood pressure tracing, followed by operation on brain		39	100–110
	March 4	Blood pressure	3	31	72–76

Body temperatures of the operated birds fixed by varying the temperatures of bird cages.

Average arterial pressure—body temperature 34–41°C.—was 99 mm.

Average arterial pressure—body temperature 28–31°C.—was 87 mm.

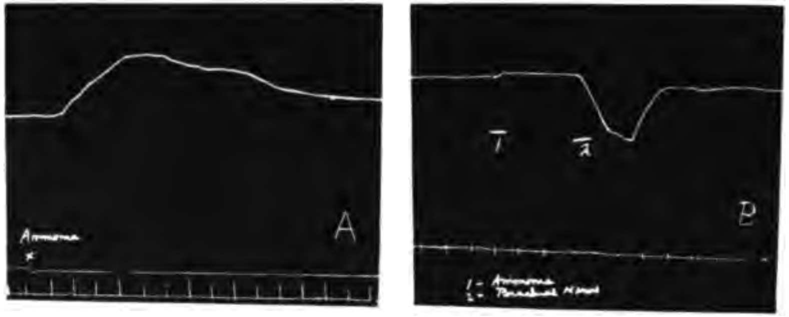

Fig. 7. Blood pressure, poikilothermous pigeons. *A:* Pigeon 169. Body temperature 31°C. Time in 15 second intervals; *x*, ammonia to nostrils. *B:* Pigeon 175. Body temperature 28°C. *1*, ammonia to nostrils; *2*, traction on brachial nerves.

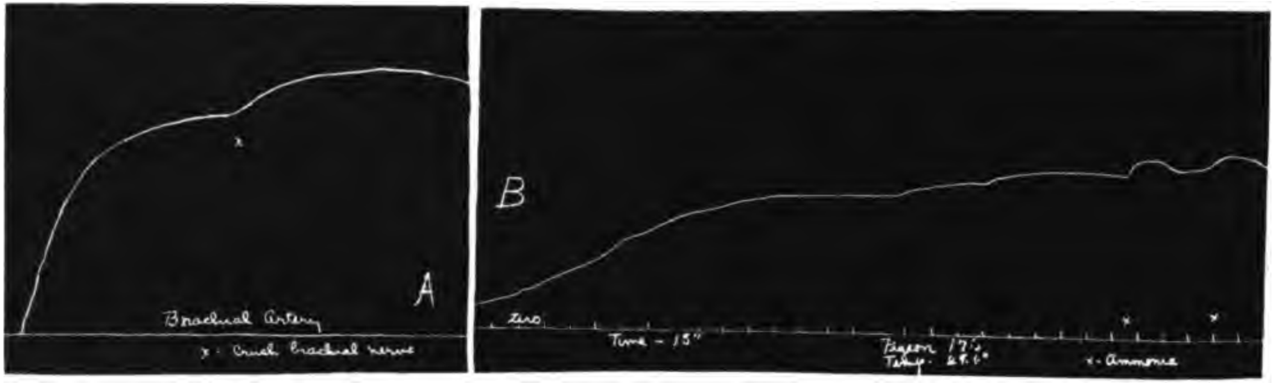

Fig. 8. Comparison of the time rates at which the pressure rises from the zero level of the manometer wholly under the influence of the arterial pressure. *A:* Normal pigeon, body temperature 39°C.; *x*, stimulation brachial nerves. *B:* Poiki othermous pigeon, body temperature 29°C.; *x*, ammonia to nostrils.

Compensation after loss of a small amount of blood occurs more and more slowly as the body temperature is more and more depressed (fig. 8). In this figure the pressure in the manometer was at zero when the artery was connected with it. The slow rise to the level of arterial pressure is in marked contrast to the quick ascent and maintenance of level in the normal bird.

Effects of localized lesions. The major part of the cerebral cortex had been destroyed by electro-cauterization in three birds a year previous to the determination of arterial pressure. All of these birds gave normal blood pressure values (table 10).

TABLE 9

Effects on arterial pressure of removing one cerebral hemisphere

NUMBER OF PIGEON	DATE	PROCEEDING	ARTERIAL PRESSURE	REMARKS
			mm.	
190	June 10	Blood pressure then operation	98–118	
	June 23	Blood pressure	96–122	Bird in good condition—eats and drinks
188	June 8	Blood pressure then operation	92–102	
	June 23	Blood pressure	80–96	Emaciated, does not eat
186	May 25	Blood pressure then operation	100–118	
	June 8	Blood pressure	108–120	Good condition, eats and drinks
187	May 25	Blood pressure then operation	94–110	
	June 7	Blood pressure	82–92	Poor condition, does not eat
	June 9	Bird dead of starvation		

In one pigeon both occipital poles of the cerebral hemispheres were removed and the dorso-median part of the thalamus cauterized without severing the forebrain bundles. Four months later the arterial pressure in this bird was normal (pigeon 118, table 10).

In one bird extensive cerebellar traumatism was done a year before the blood pressure determination. The bird regained its ability to coördinate its muscle activities in the usual way. Blood pressure determination in this bird showed a lower arterial tension (pigeon 193, table 10). At autopsy the bird was found to be tubercular.

In a series of four birds the right cerebral hemisphere only was removed (table 9). After this operation some birds promptly recover and in a few days feed themselves in a normal way. The principal obvious disturbance in such an animal is complete blindness (at least temporarily) of the opposite eye. In some cases these birds go into a condition resembling complete decerebration (probably due to vascular disturbances in the remaining hemisphere). These animals will not feed themselves, assume a decerebrate attitude and die of starvation unless fed by hand. In this series of four birds two recovered and two

TABLE 10

Arterial pressure after minor brain lesions

NUMBER OF PIGEON	DATE	OPERATION	ARTERIAL PRESSURE mm.	REMARKS
118	April 28	Occipital cortex removed; thalamus cauterized		
	August 28	Blood pressure tracing	120	Bird in good condition
72	August 17, 1918	Cortez cerebri cauterized		
	July 3, 1920	Blood pressure tracing	122–140	Bird in good condition
73	August 17, 1918	Cortex cerebri cauterized		
	July 3, 1920	Blood pressure tracing	104–148	Bird in good condition
62	February 19, 1919	Cortez cerebri cauterized		
	July 3, 1920	Blood pressure tracing	102–124	
193	May 15, 1919	Gross traumatism of cerebellum		
	July 6, 1920	Bird recovered. Blood pressure tracing	96–102	Bird is tubercular

died of starvation. The blood pressure readings in the two birds that recovered were normal. The combination of hemi-decerebration and starvation led to lower blood pressure.

It was found, therefore, that no localized cerebral or thalamic injury led to permanent depression of the arterial pressure. Complete loss of either hemispheres, or hemispheres and thalamus, leads to distinct arterial depression.

Artificial stimulation of cerebral cortex. Electric stimulation of the cortex of the cerebral hemispheres gave a slight rise in blood pressure with no movements of the skeletal muscle. Stimulation was done under light ether anesthesia. Stimulation of the thalamus causes a sharp rise in arterial pressure, but also leads to muscular activity. Whether this rise is a true vasomotor reaction or a mechanical one due to striated muscle contraction was not determined, but Sachs and others have shown that vasomotor reflexes are readily induced by artificial stimulation of the thalamic nuclei.

DISCUSSION

The results given above lead to the suggestion that the cerebral hemispheres exert a continuous tonic activity on the mechanism whereby arterial pressure is maintained. Whether this be through the vasomotor, skeletal or some other systems individually or combined is not determined. Porter's findings on curarized decerebrate rabbits indicate a tonic action on the vasoconstrictor centers. The relatively slight influence of the cerebral hemispheres on the skeletal muscle of the pigeon[1] suggests that in this case lowered blood pressure is also due to a loss of vasomotor tone rather than to changes in the skeletal muscle.

The possibility of a depression of arterial pressure due to unknown changes in metabolism, after loss of the cerebrum, can not at present be excluded. Some evidence has been presented that this alone is not sufficient to bring about the vascular changes described, witness the negative effects of starvation, inactivity, etc. These experiments however do not conclusively exclude a contributing metabolic factor

[1] There are no cortical motor points in the cerebral hemispheres of the pigeon except possibly for the eye (Ferrier). Electric stimulation of the exposed cortex in the unanesthetized pigeon causes no muscular movements. Removal of the hemispheres likewise leads to no paralysis. These facts suggest that the fall in arterial pressure is not due to secondary changes in skeletal muscle.

after brain injury. Further studies in metabolism after cerebral lesions must be made in spite of the negative results reported by various observers.

However this may be, the inference can not be avoided that any functional depression of the cerebral hemispheres should be followed by lowered arterial tension. This might be due to sleep, anesthesia or destruction (provided there be no increase in intracranial pressure). Certainly there are differences in detail of the mechanisms in each case but it is noteworthy that in each case of cerebral depression there is lowered arterial pressure. No localized cerebral vasomotor centers are postulated. Indeed localized injuries of either hemispheres or thalamus caused no change of arterial pressure. The lowering of arterial pressure described follows the loss of large amounts of cerebral substance rather than the loss of particular areas of the hemispheres or thalamus. To this extent, this report reaffirms for the vasomotor mechanism, the old teaching of Flourens that, in the bird, the effects of cerebral injury are due not to the loss of local centers but are proportional to the quantity of brain tissue rendered non-functional.

SUMMARY

A method is described for studying the blood pressure in the pigeon.

The average pressure in the brachial artery of thirty-nine normal adult pigeons, under ether anesthesia, was 118 mm. mercury. The average limits of variations, due to variations in anesthesia, were 109 to 130 mm. Pressor and depressor effects on the blood pressure may be readily induced by stimulation of the spinal nerves. Ammonia to the nostrils causes a sharp rise in blood pressure. Respiratory waves and Traube-Hering waves of blood pressure occur as in mammals. The loss of small quantities of blood is quickly followed by compensatory changes bringing the pressure back to normal.

Starvation for three to seven days does not appreciably alter the pressure in normal birds.

Removal of the cerebral hemispheres in fifteen birds led to a fall of the average arterial pressure to 99 mm. (loss of 17 per cent). This lowered pressure persisted for time intervals up to four months after decerebration and never regained the level before operation. Removal of the cerebral hemispheres and thalamus leads to a similar or greater fall in arterial pressure varying as the body temperature varies. The greater the fall in body temperature, the greater the depression of the arterial pressure.

The poikilothermous condition in the bird, following excision of the thalamus, is not primarily due to lowered arterial pressure.

In the pigeon rendered poikilothermous by combined decerebration and destruction of the thalamus, the vasomotor responses to mechanical stimulation of spinal nerves, ammonia to the nostrils, and compensatory recovery of pressure after slight hemorrhages, are all depressed or take place more slowly, varying with the depression of body temperature.

The arterial pressure is not appreciably disturbed by removal of a single cerebral hemisphere, localized lesions of both hemispheres, or localized thalamic lesion (without cerebral destruction) provided these injuries are not associated with starvation.

These experiments suggest that the cerebral hemispheres and thalamus exert a continuous tonic stimulating action on the subcortical blood pressure regulating mechanism. This action is not one of localized cerebral centers but varies according to the amount of brain substance destroyed, rather than the particular area destroyed.

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EXPERIMENTAL STUDIES IN DIABETES

SERIES II. THE INTERNAL PANCREATIC FUNCTION IN RELATION TO BODY MASS AND METABOLISM[1]

5. The Influence of Fever and Intoxication

FREDERICK M. ALLEN

From the Hospital of the Rockefeller Institute for Medical Research, New York

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The accuracy with which the metabolism of cold-blooded animals can be regulated through the temperature was one of the reasons for the attempt to produce diabetes in them at the outset of this investigation. When the production of a satisfactory type of diabetes proved impossible, the research was thrown back entirely upon mammalian experiments, where the disturbing factors are greater. Some observations were made concerning the effects of fever and of external cold.

No discussion of the literature will be undertaken, beyond reference to a review (1) of the earlier literature, and a more recent paper by Freund and Marchand (2), which show that elevation of body temperature is generally accompanied by elevation of blood sugar, but terminal collapse may be accompanied by hypoglycemia; the rise of body temperature in itself tends to increase sugar tolerance, and lowered tolerance or glycosuria are attributable to intoxication or sometimes to pancreatic damage. Infections are known to be one of the worst agencies in aggravating human diabetes. The effect of aseptic elevation of temperature seems not to have been tested. It was desired in the present research to compare several forms of infectious and non-infectious fever in their influence upon partially depancreatized dogs. The only results actually permitted by circumstances consisted in records of a number of animals which acquired chance infections, and one research concerning the gas bacillus. The observations will be classified according to the site of the infections.

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[1] The first four papers of this series are published in the American Journal of the Medical Sciences.

Distemper. For this purpose canine distemper is closely comparable to human tuberculosis. Diabetes plainly increases the susceptibility of dogs and still more of puppies to this infection, in the sense that they both acquire it and succumb to it more easily. Tuberculosis seriously lowers the food tolerance and increases the tendency to glycosuria and hyperglycemia in human patients even in the earlier non-febrile stages, and this effect becomes still greater in the febrile stage. In numerous observations in dogs covering all stages of distemper and all degrees of diabetic tendency, precisely the opposite effect has been found. It is true that distemper is characterized by early failure of appetite and digestion. The resulting emaciation constitutes a very radical undernutrition treatment, and a similarity thus exists to Joslin's frequently quoted Case R (3), in which the emaciation of tuberculosis on a regulated diabetic diet evidently improved the assimilation. But the infection has been observed in dogs with definitely known tolerance, which continued for some time to take a diet close to the limits of tolerance. There have been other examples such as dog C3-77, 1 year old, weighing 10.5 kilos, and subjected on April 13, 1916, to the removal of all but $\frac{1}{22}$ to $\frac{1}{28}$ of the pancreas (estimated remnant 1 gram).[2] Glycosuria began immediately and by April 17 had reached 2.9 per cent, fasting. It then ceased as the first signs of distemper appeared in the form of conjunctivitis. The dog refused food and wasted away in the usual manner till killed on May 7, at a weight of 6.3 kilos. The pancreas remnant weighed 1.35 grams. The islands showed very slight vacuolation in a few cells, such as might persist from the initial glycosuria, but far less than would result from 3 weeks of active diabetes. The remnant was so small and the diabetic tendency so strong that sugar-freedom on fasting would have been impossible if the infection had introduced any aggravation, but the result seemed to be identical with that in a non-infected animal.

Pneumonia. Dog B2-18, after removal of $\frac{12}{13}$ of the pancreas on December 2, developed moderate glycosuria on diets containing carbohydrate, in a cold environment. December 6 the dog was found to be unwell and febrile, but continued to eat the diet through the illness to December 9, and the glycosuria continued unchanged. Death occurred from double pneumonia on December 13. The autopsy urine contained 1.65 per cent sugar. The vacuolation in the pancreatic islands was similar to that of non-infected dogs at the same stage.

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[2] All operations were performed under ether anesthesia.

Dog C3-73 similarly underwent operation leaving $\frac{1}{10}$ to $\frac{1}{11}$ of the pancreas on April 3, and died of pneumonia on April 18. The final urine on April 17 contained heavy sugar, but death was apparently preceded by anuria.

Dog D4-72 was left with a remnant of $\frac{1}{9}$ to $\frac{1}{10}$ of the pancreas on January 12, and died of pneumonia on January 19. The appetite continued and the glycosuria was not appreciably changed with the onset of fever. Nothing was eaten after January 17. The autopsy urine still contained a trace of sugar. There was thus a diminution resembling the effect of ordinary fasting, not the great increase which usually accompanies infection in any severe human case.

Cat A1-93 was left with a remnant of $\frac{1}{5}$ to $\frac{1}{6}$ of the pancreas on January 21, and died of pneumonia on January 26. Because of refusal of food, there was only a transitory trace of glycosuria, as would be the case in a non-infected cat fasting after such an operation.

Other examples might be given in which infection failed to cause glycosuria when the removal of pancreatic tissue was not quite sufficient to produce it in a non-infected animal, and still others in which extreme prostration prevented glycosuria which must otherwise have occurred. In no instance was any evidence of aggravation of diabetes seen.

Pleurisy. Dog B2-22 had been used in another department for collection of leukocytes by intrapleural injections, and on December 8, $\frac{9}{10}$ of the pancreas was removed without knowledge of the existence of a large purulent pleurisy. Fever, malaise and other symptoms were found after the operation. The dog ate small quantities of bread and milk on December 9 and 10, and glycosuria of 2 to 3 per cent continued to December 12. Death occurred with sugar-free urine on December 13.

Subcutaneous abscesses. In connection with subcutaneous injections and other procedures a considerable number of abscesses have been observed in dogs with various degrees of diabetic tendency. The infections themselves have been of varying magnitude, from small collections causing no systemic symptoms to large ones accompanied by depression, anorexia and fever above 105°F. The organisms present were sometimes identified as staphylococci, streptococci or mixed bacilli. The same rule held as above, namely, that glycosuria might cease with fasting and prostration, or in less extreme cases might continue unchanged, but a marked aggravation such as is familiar in human cases was never seen.

Infected glands. Dog D4-92 on February 5, 1917, was subjected to removal of all but $\frac{1}{8}$ to $\frac{1}{6}$ of the pancreas. Bread and soup were eaten on February 8, and 100 grams glucose added on February 9, still without glycosuria. Thereafter nothing was eaten and remarkable symptoms of confusion and ataxia appeared, increasing on February 12 to general convulsions and suggesting rabies. The dog was chloroformed on February 13, and the brain examination was negative for meningitis or Negri bodies. The autopsy otherwise was negative except for a little creamy pus found oozing from between the pectoral muscles, leading to caseous-appearing glands in and about both axillae. The type or origin of the infection was not determined. Glycosuria remained absent.

Rabies. Several partially depancreatized dogs died of rabies. One of these was dog B2-02, which, as previously mentioned (4), had been carefully studied and was known to have latent diabetes. No glycosuria resulted in any instance. The negative results were of interest in a condition attended with such pronounced nervous excitation, and in which convulsions may give rise to very marked hyperglycemia (5).

General peritonitis. This naturally involves cessation of glycosuria in most cases because of fasting and prostration. With sufficiently large experience, examples are encountered which indicate that the infection in itself does not alter the glycosuria. Some such were described previously (6), and the following have been observed since.

Dog B2-13. November 24, 1913, removal of $\frac{14}{15}$ of pancreas. There was glycosuria of 0.25 per cent in 60 cc. of urine following operation, and 0.2 per cent in 330 cc. after eating 150 grams meat on November 27. Otherwise there was fasting and freedom from glycosuria up to death from peritonitis on November 29.

Dog B2-21. December 4, 1913, partial pancreatectomy leaving a remnant of $\frac{1}{13}$ to $\frac{1}{14}$. After bread feeding on December 5, heavy glycosuria began, and continued to death from peritonitis on December 11. Bread was eaten daily to December 9. The autopsy urine was 100 cc., with 2.85 per cent glucose. The pancreatic islands showed the slight vacuolation proper to this early stage of diabetes.

Cat A1-82. December 19, 1913, removal of $\frac{7}{8}$ of pancreas. The cat refused food but acted well and cleaned her fur up to December 22, and died of peritonitis December 23. Glycosuria began with a faint reaction on December 21, rose to 2.5 per cent on December 22, and was 4 per cent in the last 55 cc. of urine on December 23. The pancreatic islands showed incipient vacuolation in a minority of cells.

Cat A1-87. January 8, 1914, removal of $\frac{5}{6}$ of pancreas. There was slight continuous glycosuria with very little eating from January 9 to death from peritonitis on January 12. The islands were free from visible vacuolation, as would be expected in a non-infected animal with such brief and mild diabetes.

Peritoneal and pancreatic abscesses. In addition to previous examples (6), the following may be mentioned.

Dog D4-65. December 21, 1916, an Eck fistula was unsuccessfully attempted, and some sutures were left on the veins. January 2, 1917, $\frac{9}{10}$ of the pancreas was removed. The dog was lively and immediately developed glycosuria on bread feeding. This ceased on January 8, and was restored by addition of 100 grams glucose daily. On January 10 emaciation, fever and weakness first became noticeable, but the diet was still eaten without change in the heavy glycosuria. With a change of diet to 1 kilo of beef lung on January 12 glycosuria immediately ceased. Beginning January 14 food was refused, and the dog was killed January 15, at a weight of 9.8 kilos as opposed to an original 13.5 kilos. A grape-sized abscess of creamy pus at the site of the Eck operation was the only discoverable cause of death. It had not altered the course of the diabetes from what is the rule with non-infected dogs under the same conditions.

Dog D4-57. December 7, 1916, removal of $\frac{4}{5}$ of the pancreas. The usual complete absence of diabetes was demonstrated thereafter. March 1, 1917, additional tissue was removed, possibly sufficient for mild diabetes. Malaise, fever and complete refusal of food followed. Glycosuria was absent on March 2, 3 and 4, but present just before death on March 5 to the extent of 0.8 per cent in 182 cc. of urine. The plasma sugar at this time was 0.625 per cent, CO_2 capacity 69.2 vol. per cent. Autopsy showed the pancreas remnant to be riddled with small abscesses, and though there was no necrosis the inflammatory injury had evidently brought on a severe degree of diabetes which would otherwise have been lacking. Infection has never been found to produce acetonuria or other evidences of acidosis in any animal.

Dog F6-14 was subjected to removal of about $\frac{7}{8}$ of the pancreas in three successive operations. January 31, 1918, an attempt was made to produce diabetes by circulatory stasis of the remnant, as described in a later paper. Only slight and transitory glycosuria resulted on a diet of bread and soup with 100 grams glucose. February 8, operation showed an abscess containing about 5 cc. of creamy pus between the pancreas and the duodenum. The cavity was cleaned and stasis repeated. Glycosuria was still impossible to maintain, and on March 9 stasis was applied for a still longer time, no infection being found. Glycosuria was then continuous up to March 19 on bread diet with 100 grams of glucose, but ceased then on plain bread and soup feeding. March 20 the abdomen was again opened, and the pancreas was found buried in a large mass of adhesions, which when delivered outside and opened was found to contain a very large abscess. The pancreas remnant, which in its whole length formed one wall of the abscess, was much inflamed but not digested. Nothing was done except the cleaning up of the infection, and the tolerance continued exactly as before; i.e., glycosuria was absent on bread feeding and present with addition of 100 grams of glucose. On April 10 the abdomen was again opened, and a tiny abscess in the omentum appeared as the only remains of the previous infection. Stasis was again applied to the pancreas remnant, and the dog died within 24 hours, whether from infection or from pancreatic intoxication was undetermined.

The long history of this animal, with alternate presence and absence of a low grade infection, seems to prove that in this instance the infection had no important influence upon the tolerance.

Other examples of this sort might be given. There was particular interest in the cases in which diabetes was produced by inflammation instead of by simple resection, because of the supposed closer imitation of the clinical etiology. It was conceivable that inflammation might damage the islands in function as well as in structure, so as to render them more susceptible to toxic influences. The negative results raised a question concerning some fundamental difference between clinical and experimental diabetes, or a mere difference of constitutional reaction to infection on the part of man and animals. The above general observations sufficed positively to exclude any such marked aggravation of diabetes in animals as occurs regularly in human cases with the fever and intoxication accompanying infection. There remained the need of making a more exact test of the tolerance in experimental diabetes as influenced by infection, and this opportunity was afforded by the experiments with the gas bacillus reported in the next paper. These seemed to indicate that the difference between clinical and the experimental diabetes may be one of degree rather than of kind.

CONCLUSIONS

1. The serious aggravation of diabetes, which occurs almost invariably in human cases in the form of a strongly increased tendency to glycosuria and acidosis, is never seen in dogs. Even when the infection is an abscess bordering or invading the pancreatic tissue, no influence is evident beyond that explainable by direct injury of parenchyma. This contrast between clinical and experimental diabetes is very marked, but according to the more exact tolerance tests in the succeeding paper it may represent a difference of degree rather than of kind.

2. Infection and fever have also no specific influence in diminishing the diabetic tendency of dogs. Care is necessary in interpreting such observations, in order not to confuse the direct influence of fever or infection with the consequences of fasting or prostration, which tend so strongly to suppress glycosuria in dogs. One suggestion of a constitutional difference between species may be found in the tendency of human patients to acidosis and of dogs to cachexia.

3. The aggravation of human diabetes is a reaction to intoxication rather than to fever, as shown by its occurrence in the afebrile stage

of tuberculosis and by other evidence. The present observations concerning infectious fever, with the previous ones concerning the pyrexia of exercise in dogs, prove that no specific aggravation of diabetes or lowering of tolerance results from the metabolic alteration attendant upon elevation of body temperature in experimental animals.

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EXPERIMENTAL STUDIES IN DIABETES

SERIES II. THE INTERNAL PANCREATIC FUNCTION IN RELATION TO BODY MASS AND METABOLISM

6. Gas Bacillus Infections in Diabetic Dogs

MARY B. WISHART AND IDA W. PRITCHETT

From the Hospital of the Rockefeller Institute for Medical Research, New York

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In the course of this diabetic research four dogs died of gas bacillus infection. Two of these instances were merely post-operative peritonitis, with the gas bacillus predominating.

Another was dog E5-74, a bulldog mongrel, aged 5 years, in splendid condition, weighing 20.7 kilos. July 3, 1917, two-thirds of the pancreas were removed, and most of the remnant was cut off from duct communication.[1] On July 20, the remnant was subjected to circulatory stasis for 1½ hours. Glycosuria was present on bread diet with addition of 150 grams of glucose daily up to July 30, when it ceased, and the dog was turned loose in the yard with other dogs on bread diet. The behavior meantime was normal. About August 5, swelling in the neck became noticeable and the dog was slightly depressed. August 7, the swelling was much larger, and a deep abscess was opened surgically, releasing a considerable quantity of thick bloody pus containing gas bubbles. Cultures from some of the necrotic debris gave a pure growth of B. aerogenes capsulatus. Death occurred August 9, after still greater invasion of the neck. There was no glycosuria or vacuolation of pancreatic islands, though both these conditions might have been prevented by the terminal emaciation and cachexia.

Dog B2–49, a female mongrel aged 3 years, in medium nutrition at a weight of 25.4 kilos, underwent partial pancreatectomy on March 27, 1914, leaving a remnant of ¼ to ⅕ about the main duct. As previously mentioned (1), prolonged carbohydrate over-feeding was used in the attempt to break down tolerance, 300 or 400 grams glucose being added to the diet of bread and soup daily. There was neither glycosuria, diarrhea nor any evident ill-health, until the animal was unexpectedly found dead on May 9. There were adhesions in the right pleura, from supposedly sterile intrapleural injections in another department long before the animal was taken for diabetic work, and these probably furnished the start of the infection. Gas bacilli were found abundantly in smears and cultures from the principal viscera, seemingly alone. The greatest change was in the spleen, which was blown up to resemble a lung. The pancreas remnant was normal and free from vacuolation. In other words, neither the prolonged sugar feeding nor the infection produced any change in either islands or acini in this non-diabetic animal.

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[1] All operations were performed under ether anesthesia.

A study of gas bacillus infections was in progress at this time under the direction of Dr. Carroll G. Bull. As gas bacillus infections are rare in dogs, it was decided to follow up the above accidental observations by experiments upon diabetic dogs with a view to two questions; first, whether such animals are abnormally susceptible to such infections by reason either of the excess of circulating sugar or a specific diabetic lowering of resistance; second, whether an aggravation of the diabetes is demonstrable by such infections. The conditions were favorable for both problems; for the first problem because the growth of the gas bacillus is notably favored by the presence of sugar, and some test was thus afforded of the theory of excess of sugar as the cause of diabetic susceptibility to infection; for the second problem because of the proof (2) of the production of a soluble toxin by the gas bacillus, so that a systemic effect capable of influencing the diabetes might be expected from a local infection. Accordingly experiments with intramuscular injections of pure cultures of the Welch bacillus were performed upon three diabetic dogs. The dosage used was intended to produce the maximum possible local effects and general intoxication without excessive prostration. Still larger doses might have overwhelmed the animals suddenly and completely, but would thus have demonstrated nothing of value for either bacteriology or diabetes.

In the first experiment (table 1) glycosuria practically ceased with the anorexia accompanying infection on September 12, as usual with dogs and in contrast to the usual aggravation of symptoms in human patients with infection. Nevertheless a lowering of tolerance was shown by the heavier glycosuria when the diet was taken on September 13. Illness and fasting again resulted in sugar freedom after the injection of September 14, but a more marked lowering of tolerance was evident in

TABLE 1

Dog E5-88. Male; Welsh terrier mongrel; old but strong, in excellent nutrition; weight 11.25 kilos. August 24, 1917, removal of pancreatic tissue weighing 18 grams. Remnant about main duct estimated at 1.6 grams ($\frac{1}{12}$ to $\frac{1}{13}$). The diabetes was checked by undernutrition and fasting, so that at the time of the experiments the dog weighed 9.5 kilos and took a bread and soup diet with very slight glycosuria.

DATE	RECTAL TEMPERATURE	URINE		REMARKS
		Volume	Glucose	
1917	°*C.*	*cc.*	*per cent*	
September 10		660	0.3	Bread and soup diet
September 11	38.7	450	0.4	
September 12		510	Trace	At 10:45 a.m. injected 0.1 cc. per kilo of broth culture of Welch bacillus intramuscularly right thigh. Marked local edema and swelling. Dog depressed; ate nothing
10:00 a.m.	38.8			
1:15 p.m.	39.7			
3:30 p.m.	40.7			
5:30 p.m.	40.4			
September 13				Thigh still swollen. Dog unwell but took entire diet, part forcibly
9:00 a.m.	39.7	880	2.6	
September 14				9:30 a.m., injected 0.3 cc. per kilo of broth culture of Welch bacillus intramuscularly left thigh. Much local swelling. Dog ill and feverish; refused diet but ate a little meat
9:00 a.m.	38.3	670	2.8	
September 15	39.8	320	1.4	Refused all food
September 16		425	0.3	Refused all food. Great edema and crepitation, extending into scrotum
September 17		510	Trace	Refused food
September 18		650	0	Refused food
September 19		420	0	Refused food
September 20		400	Trace	Ate a trifle of meat and bread
September 21	39.5	450	0	Ate very little of meat
September 22		480	Trace	Ate more meat. Much swelling and gas in leg
September 23		460	0.6	Acting better. Ate more meat
September 24		400	1.2	Ate some bread and meat
September 25		500	2.1	Ate full diet
September 26		700	2.9	Ate full diet
September 27		630	3.4	Ate full diet
September 28		610	3.1	Ate full diet
September 29	38.8	1200	2.2	Both thighs have discharged necrotic material, leaving granulating ulcers. Dog lively and vigorous

the glycosuria from meat alone on September 22 and 23, and the heavier glycosuria thereafter on the regular bread diet.

Dog E5–89. Male; mongrel; age 3 or 4 years; good condition; weight 14 kilos. August 24, 1917, removal of pancreatic tissue weighing 25 grams. Remnant about main duct estimated at 1.6 gram ($\frac{1}{16}$ to $\frac{1}{17}$). Severe diabetes being thus produced, the glycosuria was raised to a maximum by a diet of bread and soup with 100 grams of glucose daily.

September 7, at a weight of 12.6 kilos, 0.25 gram additional pancreatic tissue was removed for microscopic examination.

September 14, at the same weight, 0.1 cc. broth culture of Welch bacillus per kilo was injected intraperitoneally, in order to test whether under these conditions of maximum glycosuria and hyperglycemia infection would be possible. The rectal temperature rose within an hour to 39.4°C. After 6 hours it was 39.5°, and the next morning 39.6°. It then subsided, and after one day of slight malaise the dog continued to eat his diet. The glycosuria continued unchanged except for a diminution on the one day of anorexia.

September 24, an injection of 0.3 cc. of broth culture per kilo was given intramuscularly in one thigh. The usual local and general symptoms occurred in intense form. September 29, with very large swelling and gas formation present in the leg, a blood culture was taken and proved negative. The dog regained a little appetite, taking small amounts of meat and bread daily, but great anemia was shown by blood examinations, the corpuscle volume being only 10 to 12 per cent. Death occurred October 5. Glycosuria remained heavy throughout, including the autopsy urine. The gross autopsy showed no visceral changes suggestive of gas bacillus invasion. Cultures of blood and tissues were also negative for this organism.

The pancreas remnant, normal in appearance and consistency, weighed 1.7 grams. Microscopically, the tissue removed August 7 showed a very early stage of vacuolation of islands. The remnant at autopsy showed a late stage of the process; islands were scarce and small, and the great majority of the cells (probably all of the beta cells) were maximally vacuolated.

In this experiment the production of a general infection with the gas bacillus proved impossible notwithstanding the severe diabetes and intense glycosuria. The intraperitoneal injection failed entirely. The intramuscular injection caused extensive sloughing which destroyed most of the musculature of the limb, but death resulted only from the immediate and subsequent toxic effects and not from systemic invasion.

Dog E5–90. Male; mongrel, age 3 or 4 years; medium nutrition; weight 10.25 kilos. August 24, 1917, removal of pancreatic tissue weighing 20.3 grams. Remnant about main duct estimated at 1.6 gram ($\frac{1}{13}$ to $\frac{1}{14}$). On bread diet there was a diminishing glycosuria which ceased August 31, probably because of hypertrophy of the pancreas remnant, which subsequently at autopsy was found to weigh 5.4 grams. Beginning September 8 the addition of 100 grams glucose restored a heavy glycosuria, and the tolerance was brought down so as to produce a permanent mild diabetes. During October sugar freedom was maintained on a diet of 500 grams lung and 100 grams suet, except for occasional days on which it was proved that bread and soup diet would promptly bring back a mild glycosuria.

October 25, an intravenous glucose tolerance test was performed, by injection of 25 cc. of 10 per cent solution of Merck anhydrous glucose every 15 minutes (1 gram per kilo per hour, on 10 kilos weight) for 3 hours, according to the method described elsewhere (3). Catheterization was performed and blood samples taken before the first injection and at hourly intervals thereafter as shown in table 2.

October 26, 4 cc. of a heavy broth culture of the Welch bacillus were injected intramuscularly in the right thigh. The rectal temperature rose to 41.1°C. that evening and was 39.6° the next morning. The dog refused food and there was no glycosuria. By October 31 there was partial recovery and part of the diet was eaten. The weight had fallen from 10 kilos to 9.75.

TABLE 2

BLOOD			URINE						REMARKS
Plasma sugar			Volume			Glucose			
October 25	November 1	December 18	October 25	November 1	December 18	October 25	November 1	December 18	
per cent	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*	*grams*	*grams*	*grams*	
0.164	0.133	0.122				0	0	0	Before injection
0.333	0.400	0.384	5	41	14	0.034	0.450	0.320	End of 1st hour
0.323	0.400	0.500	14	42	50	0.080	1.150	2.000	End of 2nd hour
0.213	0.216	0.455	95	14	26	Faint	0.360	0.680	End of 3rd hour
0.081			400	150	30	Slight	0.690	0	Next morning

November 1, an intravenous glucose test was performed, identical with the dosage on October 25. A lowering of tolerance was indicated by both the blood and urine analyses.

November 14, 4 cc. of the gas bacillus culture were injected in the other thigh. Local edema, gas formation and necrosis occurred as before, but the general symptoms were less. The temperature on the morning of November 15 was 38.8. The dog ate well and showed a spontaneous glycosuria of 2.15 per cent in 340 cc. urine. The following day it was 0.48 per cent in 530 cc. urine, and then disappeared.

Later the dog was unwell and ate poorly, probably on account of secondary infection of the sloughing area in the leg. No further glycosuria developed, and by December 18 the animal was again in good general health at a weight of 10 kilos, though a large open ulcer was still present.

December 18, the animal was given the same intravenous glucose injections as before. A reduced tolerance was still indicated, either because of the ulcer or because the lowering due to infection was permanent, as it is in many human cases.

An accidental or spontaneous fall of tolerance is probably excluded by the fact that the dog was kept on the lung and suet diet till March 27 without glycosuria. He was then used for other experiments, and no further tolerance test was made.

CONCLUSIONS

1. Intramuscular injections of pure cultures of B. aerogenes capsulatus produced local necrosis and gas formation in partially depancreatized diabetic dogs. Systemic or peritoneal infection was not obtained. The observations failed to indicate any lowering of resistance in these animals due either to the diabetes itself or to the excess of sugar in the body fluids. The latter point is further emphasized by the fact that the reactions were essentially similar in the first dog with mild glycosuria, in the second dog with heavy glycosuria, and in the third dog free from glycosuria. These results agree with the general experience that such animals ordinarily bear operations well and their wounds heal normally.

2. A lowering of tolerance by infection was demonstrable both by feeding and by intravenous glucose tests. Though this influence is less in animals than in human patients, the difference seems to be one of degree rather than of kind.

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STUDIES IN EXPERIMENTAL TRAUMATIC SHOCK

I. The Basal Metabolism

JOSEPH C. AUB

With the Technical Assistance of K. Sandiford

From the Laboratory of Physiology in the Harvard Medical School

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The marked loss of body temperature is one of the most striking features of traumatic shock. To study this, as well as to investigate the relationships of metabolism and ventilation to the sudden changes in blood pressure, was the purpose of these experiments. The problem was approached through the gaseous exahange.

The literature upon this subject is very meager. Guthrie (1), reported no consistent findings in either O_2 absorption or CO_2 output with animals under ether anesthesia. Henderson, Prince and Haggard (2), in a preliminary note, mention a marked drop in metabolism in two dogs in shock, but give no details of experiments. Roaf (3), working on decerebrate cats, states that his experiments tend to show that fall of blood pressure does not markedly reduce the production of CO_2.

Methods. Cats were used that had not eaten for 24 hours. They were anesthetized by urethane given by mouth, 8 cc. of a 25 per cent solution per kilo of body weight, and only when fully anesthetized were they stretched out on an animal board. The temperature was recorded through a rectal thermometer graduated to tenths, and was kept as nearly constant as possible by means of an electric heating pad.

The operation consisted of inserting a trachea cannula and cannulae in two arteries, usually both carotids, and also usually one in the external jugular vein. One carotid cannula was then attached to a mercury manometer and blood pressure tracings begun. In some experiments 10 cc. of arterial blood were now removed; in most cases blood was taken only after several respiration samples had been obtained.

The inspired air was room air. The samples of expired air were obtained in two 8-liter copper spirometers. The valves used were Tissot valves, attached directly on the T-shaped glass tracheal cannula. The air sample was promptly withdrawn from the spirometer and preserved under pressure in the usual type of glass sampling tube. Gas analyses were made in the Haldane apparatus, and careful checks of room air were made before samples were analyzed. Urinary nitrogen determinations were not made (4).

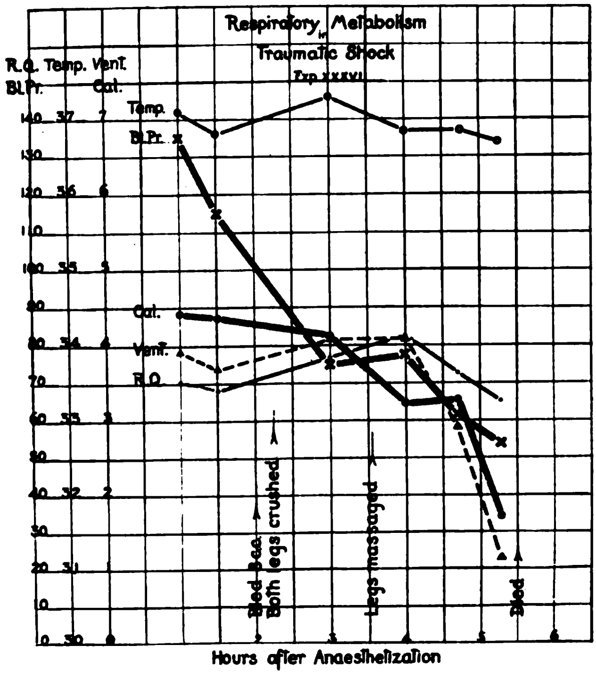

Fig. 1. Vent. = Ventilation volume per minute in 100 cc.
Cal. = Total calories per hour.
R. Q. = Respiratory quotient.

To produce shock the thigh muscles of both hind legs were thoroughly crushed (5). When the blood pressure fell below 70 mm. Hg. systolic and stayed below that level, the animal was considered to be in a state of shock. No attempt was made to measure blood flow, as all extra manipulation was rigidly avoided.

The experiments may be grouped as follows:

I. Normal controls. Table 1.

II. The simple traumatizing of muscle tissue and the study of respiratory metabolism before and after the resulting drop in blood pressure. Tables 3 and 4.

III. The production of traumatic shock and the subsequent raising of the blood pressure by transfusion with cat's blood. Table 5.

IV. The effect on metabolism of hemorrhage when it alone caused a marked fall of pressure. Table 2.

V. The production of a low blood pressure without shock by increasing pericardial pressure. Table 6.

Discussion. Table 1 shows that the metabolism following urethane anesthesia when given by mouth remains quite constant for 4½ hours at least. It may then fall to a lower level. Raeder (6) kept rabbits alive for over 3 days by administering urethane subcutaneously. He came to the conclusion that the total metabolism fell only about 2 per cent an hour, and that it was a satisfactory anesthetic to use in studying respiratory metabolism.

The level of the basal metabolism during shock has fallen in all but one case below the value found before the muscles were crushed. In six cases of mild shock (table 3) the average reduction in calories was −19 per cent, and in eight cases of severe experimental shock (tables 4 and 5) the average fall was −30 per cent. This average does not include experiment LIII in which the metabolism rose. Likewise in five experiments in which pericardial pressure was increased (table 6) and the blood pressure so reduced, there was a prompt drop (fig. 2) to an average of −31 per cent below the former height. In general the "critical level" of blood pressure for the metabolism, as with the development of diminished alkaline reserve, is at 75 or 80 mm. Hg. At that level the metabolism may be within normal limits or it may be considerably reduced. Usually when a normal value is found, the blood pressure has been stationary or rising; but when the metabolism figures are reduced the blood pressure is falling. With a pressure below 75 mm. Hg., the calorie production has, with but one exception, been reduced.

While the reduced blood pressure undoubtedly has a great deal to do with the fall of metabolism, it is probably not the whole story. Experiment XXXIV, table 2, shows that a low blood pressure, following hemorrhage alone, may be associated with only a slight drop in metabolism. With a blood pressure of 55 to 62 mm. Hg. immediately after bleeding the metabolism was only −10 per cent; and in the third period, although the rising blood pressure had been below 80 mm. Hg. for 45 minutes, the metabolism was only −1 per cent. So also in the last period after the pressure had been 50 mm. Hg. for 20 minutes, but was rapidly rising at the time the period was taken, the metabolism was only −7 per cent.

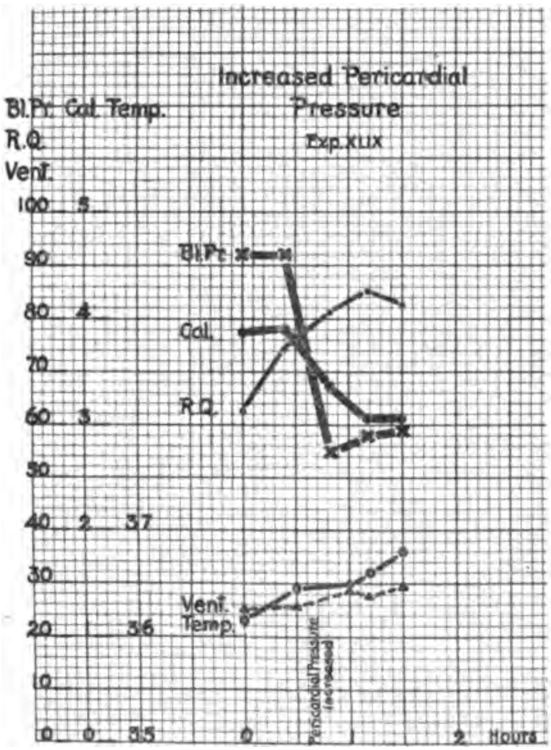

Fig. 2. Vent. = Ventilation volume per minute. The figure should be multiplied by ten.

There are also examples of blood pressures above the critical level associated with reduced metabolism. In experiment XXXIII, table 2, the second period shows a sudden drop of 22 per cent in metabolism 20 minutes after bleeding 15 cc., while later a return to normal limits occurred even though muscle injury was done. This bleeding only caused an immediate drop in blood pressure from 110 to 90 mm. Hg.,

TABLE 1

Respiratory metabolism; controls

EXPERIMENT NUMBER, WEIGHT, DATE	TIME OF PERIOD	RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY RATE PER MINUTE	RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	TIME OF ANESTHETIZATION	REMARKS
		CO_2	O_2		Calories	Variation						
		cc.	*cc.*			*per cent*		*cc.*	°*C.*	*mm. Hg.*		
Experiment XXVI, 3/28/19, 3200 grams, ♂	11:35	19.4	28.3	0.69	7.83		62	583	38.0	95–105	10:52	
	12:07	21.2	27.9	0.76	7.90			615		110		
	12:50	20.2	28.6	0.71	8.03		44	587	37.0	120–110		
	2:00	19.9	27.7	0.73	7.78	−1	46	606	36.5	110		
	3:00	22.0	28.8	0.76	8.18	+4	*	694	36.8	115–120		
	4:00	19.7	26.1	0.75	7.38	−6	46	474	36.8	120		
	5:10	18.7	26.6	0.70	7.45	−5		447	37.6	110		6 hours, 20 minutes after anesthesia
Experiment XXVII 3/31/19, 3100 grams, ♂	12:23							478	36.8	160	11:30	Blood CO_2 comb: P. 47.1
	1:23	19.8	25.9	0.76	7.35		20	369	36.4	150		
	2:25	19.8	26.1	0.76	7.39			427	36.6	130		
	3:30	18.7	25.8	0.72	7.25	−2		366	37.0	135		
	4:25	18.9	25.5	0.74	7.19	−2		342	37.0	135		Blood CO_2 comb. P. 53.8
	5:25	17.8	24.4	0.73	6.84	−7	27	328	36.5	115		6 hours after anesthesia
Experiment XLVI, 7/15/19, 1800 grams, ♂	11:03	12.1	14.1	0.85	4.10		51	483	36.4	108	10:10	
	11:22	11.3	14.8	0.77	4.19		53	457	36.4	106		
	12:07	9.7	13.3	0.73	3.73	−10	48	357	36.6	95		
	1:15	10.2	13.8	0.74	3.88	−6	45	414	36.8	102		
	2:02	10.1	13.5	0.77	3.81	−8	48	376	36.8	110		
	3:33	8.7	11.7	0.75	3.30	−20	44	350	36.2	100–90		5 hours after anaesthesia
	4:19	8.6	12.2	0.71	3.43	−18	45	380	36.4	100		
	5:11	8.1	11.4	0.71	3.20	−23	43	357	36.8	95		7 hours after anesthesia

Experiment LIX, 12/12/19, 3500 grams, ♀	11:09	17.0	24.6	0.69	6.91			659	38.2	107	9:50	
	11:30	18.5	25.2	0.73	7.07		44	809	38.0	105		11:50–12:00, Bled 25 cc. and transfused with 30 cc. citrated blood
	3:02	13.3	21.1	0.63	5.92	−15	36	443	38.0	100		
	3:25	14.8	22.0	0.67	6.15	−12	38	492	37.9	106		5½ hours after anesthesia
Experiment L, 3/17/20, 3000 grams, ♂	2:00	18.6	26.9	0.69	7.55			577	37.4	100	1:00	Cat had severe dyspnea before observations started
	2:20	19.5	25.3	0.77	7.18		36	554	37.3	100		
	3:00	19.1	23.5	0.81	6.75	−8	36	540	37.3	90		2:10, Blood samples taken, and blood replaced by transfusion
	4:00	19.1	24.9	0.77	7.08	−4	36	524	37.6	94		
	4:57	19.3	25.1	0.77	7.13	−3	36	547	37.6	115		
	5:36	17.6	24.6	0.72	6.89	−6	36	455	37.5	122		
	6:00	17.0	24.0	0.71	6.73	−8		409	37.5	112		
Experiment LVIII, 4/27/20, 2900 grams, ♂	12:47	14.5	19.3	0.75	5.46		24	594	37.4	100	11:35	1:00, Cat vomited
	1:11	15.1	21.7	0.70	6.10		22	474	37.5			2:10, Bled 25 cc. and blood replaced by transfusion.
	2:25	18.8	25.0	0.75	7.07	+21	20	595	37.5	110		
	3:34	17.4	24.3	0.72	6.81	+18	32	548	37.6			Cat vomited
	4:15	17.0	23.4	0.73	6.56	+13	32	525	37.9	120		3:20, Cat vomited
Experiment LXIX, 5/27/20, 2700 grams, ♀	12:25	18.4	23.6	0.78	6.71			465	37.8	127	11:15	12:55, Bled 25 cc. and blood replaced by transfusion
	12:40	14.3	19.6	0.73	5.51		18	362	37.5	118		
	1:20	15.3	20.3	0.75	5.73		24	396	37.9	106		
	2:27	15.5	20.4	0.76	5.78	−3	27	417	37.9	95		Blood clotted repeatedly in cannula during experiment
	3:06	17.2	21.9	0.78	6.25	+5	28	473	38.0	104		
	3:43	14.0	20.0	0.70	5.60	−6	22	399	38.0	104±		
	4:10	14.1	19.1	0.74	5.38	−10	30	461	38.1	110±		
Experiment LXX, 6/1/20, 3000 grams, ♀	11:37	14.2	21.5	0.66	6.03		22	467	37.4	144	10:30	
	12:47	18.6	25.1	0.74	7.08		40	700	37.6	138±		
	1:34	18.5	25.0	0.74	7.03	+7		666	37.7	145		
	2:15	18.2	23.4	0.78	6.67	+2	44	660	38.4	140±		Cat overheated
	3:45	19.1	28.0	0.68	7.85	+20	40	719	37.7	156		

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* Dyspnea.

TABLE 2

Respiratory metabolism; hemorrhage

EXPERIMENT NUMBER, WEIGHT, DATE	TIME OF PERIOD	RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY RATE PER MINUTE	RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	TIME OF ANESTHETIZATION	BLOOD CO_2 COMBINING POWER	REMARKS
		CO_2	O_2		Calories	Variation							
		cc.	*cc.*			*per cent*		*cc.*	*°C.*	*mm. Hg*			
Experiment XXXIV, 4/19/19, 2700 grams, ♀	11:15	14.7	20.4	0.72	5.73		44	523	36.2	102	9:45	48.3	Experiment shows effect of hemorrhage alone.
	11:35	13.0	18.4	0.71	5.15	−10	40	464	36.3	55–62			Period taken 5 minutes after bleeding 17 cc.
	12:15	14.4	19.8	0.71	5.65	−1	47	482	36.3	75–80			
	12:45	15.2	19.1	0.80	5.45	−5	46	536	36.4	82			
	1:45	15.0	20.2	0.74	5.69	−1	51	506	35.6	110			Blood pressure 50, 5 minutes before last observation. Period taken 20 minutes after bleeding
	2:30	14.8	18.6	0.80	5.32	−7	40	637	35.8	65–85		52.0	
Experiment XLIII, 5/15/19, 2600 grams	12:12	13.0	19.5	0.67	5.47		28	3402	37.1	130–120	11:30		Spontaneous drop of blood pressure
	12:40	10.9	16.6	0.66	4.66	−15	29	3080	37.0	95			
	1:5	11.8	17.1	0.69	4.80	−12	32	3364	37.0	85			1:30, Bled 10 cc. (pressure below 70 one hour). No trauma
	3:2	11.7	16.8	0.70	4.70	−13	29	4087	37.1	80			

Experiment XXXIII, 4/15/19, 3000 grams, ♂	10:50	18.4	25.3	0.73	7.10		38	440	35.9	115	9:35	43.5	
	11:37	16.5	19.1	0.86	5.55	−22	34	364	35.6	95			Bled at 11:15, 15 cc.
	12:34	17.8	23.1	0.77	6.56	−8	28	407	35.3	78–70			
	1:38	17.9	23.3	0.77	6.63	−7	32	469	36.0	82–84			Legs smashed 12:00–12:08
	2:3	18.1	23.0	0.77	6.68	−6	35	544	35.7	80–83			
	2:37	17.1	21.4	0.80	6.15	−13	36	559	35.9	80		43.5	
Experiment XXXV, 4/22/19, 2400 grams	12:0	14.4	22.0	0.66	6.16		30	482	36.7	105	11:0		Controls vary 14 per cent. Average taken
	12:20	14.7	18.5	0.80	5.28		25	461	36.6	90			
	12:45	14.2	16.7	0.85	4.85	−15	30	533	36.6	60–67		43.3	12:40, Bled 10 cc.
	1:47	14.4	15.4	0.94	4.56	−20	46	629	36.5	75			Effect of hemorrhage
	2:47	16.8	16.2	1.04	4.54	−21	46	1433	36.5	78–63			2:12, Both legs smashed. Traube-Hering waves developed at once
	3:48	10.6	13.1	0.81	3.76	−34	40	670	36.7	50		31.9	Cat died at 4:20 in profound shock

and at the time of observation the blood pressure was 95. It was, however, not rising. In experiment XXX, table 3, where blood pressure fell from 170 to 108 mm. Hg. after severe trauma, the metabolism still fell 14 per cent and 29 per cent; and in experiment XXXI, table 3, even though blood pressure after trauma was 85, the heat production was reduced −21 per cent and −24 per cent. The temperature in these last two experiments, however, was not satisfactory. Likewise in experiment XXXII, the metabolism fell 23 per cent even though the blood pressure was 100. In experiment XXXVI, table 4, fourth period, though the blood pressure was 80–75 in an animal rapidly developing shock, the metabolism was −26 per cent of the normal level. The explanation of these differences is obscure. After hemorrhage the blood pressure may be low for a time without marked drop in metabolism. After muscle injury the metabolism may be reduced before a great fall in blood pressure has occurred. Possibly the observations of Gesell (7) will satisfactorily account for these facts. He found that in the early stages of shock from tissue abuse there is usually a marked reduction of the "volume flow" of blood in peripheral organs, although blood pressure is only little changed, and he reports one instance of increased volume flow after hemorrhage though the blood pressure was falling. The *volume flow of blood* determines the oxygen delivery to the tissues, and this may vary to some degree without corresponding variation in blood pressure.

The reduction of blood pressure by increasing pericardial pressure, as described by Cannon (8), is due to mechanical venous obstruction, and is similar in its action to the methods described by Janeway and Jackson (9) and by Erlanger and Gasser (10). When the blood pressure is reduced by this procedure, the metabolism, as calculated from the respiratory gases, shows a marked prompt reduction to the level found with similar blood pressures in experimental shock (table 6). The respiratory quotient is also similar in that it rises. The prompt reduction of metabolism by this method of merely hindering the venous return to the heart is evidence that some mechanical factor such as retarded blood flow is the cause of the reduced metabolism. This is emphasized by the rapidity of the appearance of the reduction, which by this method seems to occur without delay.

The rapid appearance of diminished alkaline reserve in shock, as shown by the lowering of the blood CO_2 combining capacity, should not reduce metabolism but, if anything, should tend to raise it slightly, as shown by studies (11) in conditions where a similar drop in the reserve may occur.

A feature of the results is the rather low average respiratory quotient. In forty-seven observations in Raeder's publication (6) and in sixty-two normal observations in this series, the average respiratory quotient was the same—0.75. Wilenko (12) in ten periods with cats partly anesthetized by 1 gram urethane per kilo by mouth, had an average respiratory quotient of 0.79 in his controls. This rather low value, as also the work of Underhill (13), and the presence of a hyperglycemia (paper III), all suggest a decreased oxidation of carbohydrate under urethane anesthesia. This point will be more fully discussed in a future publication. Here it is sufficient to say that the control experiments demonstrate that the height of the basal metabolism remains constant under urethane for 4½ hours. Inasmuch as we are dealing with relative changes in each animal, the low respiratory quotient in the control periods does not affect the eventual conclusions. The tendency as shock develops has been for the respiratory quotients to rise—the average for twenty-one observations being 0.81, an effect, probably, of increased ventilation and the resulting pumping out of CO_2.

Having the animal anesthetized makes the determination of the basal metabolism a great deal simpler, for voluntary movements and emotional excitement are removed as factors which might raise the metabolism. The abnormality of some of the respiratory quotients reported here could practically all be traced to irregularities in breathing just prior to or during the observation. A period of hyperpnea previous to the observation gave a low respiratory quotient, and with hyperpnea during the period, the respiratory quotient was always elevated. In cats the breathing under urethane is apt to vary in quantity rather markedly and, as a result, no great stress may be laid upon respiratory quotients. Then, too, there is the factor of rapidly decreasing alkaline reserve with the marked fall of blood pressure, as shown by Cannon (14), and whether this is due to an accumulation of lactic acid or to a disappearance of alkali into the tissues, the effect would probably be a temporary pumping out of extra CO_2 into the expired air. The effect of this change in balance might well affect the dissociation curves of hemoglobin for oxygen and for CO_2 (15). With the low blood pressure found in shock, the effect on the exchange of substances between blood and tissue fluids may be considerably disturbed, and these factors might distort the relationship between the O_2 absorbed and the CO_2 given off in the lungs. As a result, little stress may be laid on the respiratory quotients obtained, because of the

TABLE 3

Respiratory metabolism in shock; mild shock

EXPERIMENT NUMBER, WEIGHT, DATE	TIME OF PERIOD	RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY RATE PER MINUTE	RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	TIME OF ANESTHETIZATION	REMARKS
		CO_2	O_2		Calories	Variation						
		cc.	*cc.*			*per cent*		*cc.*	*°C.*	*mm. Hg.*		
Experiment XXVIII, 4/2/19, 3100 grams	12:30	23.3	27.1	0.86	7.87		56	857	37.4	100–90	11:00	
	1:00	21.3	25.0	0.85	7.25		78	762	37.2	85		One leg crushed at 1:10 mildly
	2:30	19.6	26.9	0.73	7.61	+1	166	1287	38.2	80		Cat overheated
	3:50	16.5	24.0	0.69	6.72	−10	145–90	874	37.3	75		No severe shock developed
Experiment XXX, 4/7/19, 4000 grams, ♂	11:15	28.5	37.4	0.76	10.59		175	1823	37.5	165	10:10	
	11:40	31.0	39.4	0.79	11.23	+6	195	2400	37.3	150		11:30, Bled 15 cc.
	12:12	27.6	33.8	0.82	9.38	−14	146	2046	36.9	150		12:00, Both legs smashed
	2:00	24.7	32.6	0.76	9.33	−14	116	1504	36.9	112		1:40, Both legs resmashed
	2:50	22.2	26.9	0.83	7.74	−29	80	1120	36.5	108		No real shock developed
Experiment XXXI, 4/10/19, 3300 grams	10:33	20.8	24.9	0.84	7.20		22	509	36.5	150	9:42	Regulation of temperature bad
	11:20	18.6	22.6	0.83	6.51		28	475	36.5	95–100		Legs smashed 11:47 and 12:13
	11:55	17.3	22.0	0.79	6.29	−8	37	484	36.0	85–100		
	12:54	16.3	20.2	0.81	5.78	−15	34	476	35.4	70–65		Suggestive of shock
	1:45	15.3	18.7	0.82	5.44	−21	43	462	35.1	85		Recovering spontaneously
	2:27	13.7	18.5	0.75	5.21	−24	45	568	35.7	85		Temperature low
	3:22	15.6	18.3	0.85	5.30	−23	52	725	36.0	76		

Experiment XXXII, 4/12/19, 2700 grams	12:24	15.2	22.6	0.68	6.33		22	408	36.3	135	11:15	
	12:44	19.0	24.5	0.78	6.96	+10	22	496	36.2	135		1:07, Right leg smashed
	1:17	16.8	22.5	0.75	6.36	−4	15	386	36.2	130		1:40 and 2:12, Left leg smashed
	3:02	16.4	17.2	0.95	5.13	−23	32	627	36.2	100		No shock pressure but metabolism fell
Experiment XXXIX, 5/5/19, 2800 grams, ♂	11:35	17.2	23.5	0.73	6.61		29	424	37.5	135	10:45	
	11:55	16.1	22.5	0.72	6.30	−5	27	389	37.4	120		12:22, Bled 9 cc. Blood pressure fell to 80; rose rapidly
	12:45	16.1	21.5	0.75	6.07	−6	19	467	37.9	100–95		
	1:53	15.3	19.8	0.77	5.61	−13	28	547	37.4	80–87		1:05, Both legs smashed; shock just developing
Experiment LVII, 12/10/19, 4600 grams, ♀	12:05	22.3	31.0	0.72	8.70		18	613	38.9	128	10:30	
	12:25	21.6	30.9	0.70	8.67		20	608	38.8	120		1:10, Left leg smashed
	2:48	22.1	27.5	0.80	7.87	−9		950	38.3	73		Very mild shock
	3:11	21.1	24.5	0.86	7.11	−18	29	1241	38.6	72		Blood pressure never below 70

TABLE 4

Respiratory metabolism; severe shock

EXPERIMENT NUMBER, WEIGHT, DATE	TIME OF PERIOD	RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY RATE PER MINUTE	RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	TIME OF ANESTHETIZATION	BLOOD CO₂ COMBINING POWER	REMARKS
		CO₂	O₂		Calories	Variation							
		cc.	*cc.*			*per cent*		*cc.*	*°C.*	*mm. Hg.*			
Experiment XXXVI, 4/26/19, 2400 grams	11:48	11.1	15.8	0.70	4.44		40	391	37.1	135	10:45		Bled 8 cc. at 12:55
	12:20	10.6	15.6	0.68	4.38		36	369	36.8	100–130			Both legs smashed 1:00–1:05
	1:45	11.1	14.4	0.77	4.10	−7	34	408	37.3	75			
	2:45	9.4	11.4	0.82	3.23	−26	36	409	36.9	80–75			Traube-Hering waves
	3:30	8.2	11.3	0.73	3.28	−26	30	292	36.9	60–63			Traube-Hering waves
	4:08	4.0	6.1	0.65	1.71	−61	19–13	1162	36.7	55–40			Cat died 4:19 as period ended
Experiment XXXVII, 4/28/19, 3600 grams, ♂	1:10	19.8	26.5	0.75	7.48		28	470	36.5	152–140	12 N		1:55, Bled 10 cc. without loss of blood pressure
	1:30	22.2	28.3	0.78	8.06	+8	32	493	36.6	110–120			
	2:00	21.6	28.9	0.75	8.16	+5	27	546	37.0	130		41.4	2:25–3:25, Smashed both legs
	3:35	21.1	25.7	0.82	7.39	−5	26	594	37.5	110			
	5:50	15.6	18.5	0.84	5.35	−31	25	464	36.5	67–60		27.1	Profound shock just developing. 6:20, Cat died
Experiment XXXVIII, 5/1/19, 2400 grams	11:45	13.4	17.7	0.76	5.00		30–26	362	36.2	140	11:00		
	12:17	13.5	18.0	0.75	5.08		28–50	377	36.3	100			1:00, Bled 8 cc.
	1:12	11.5	16.5	0.70	4.63	−8	30	400	36.2	80			1:35 and 1:55, Both legs smashed. Shock developing
	2:22	16.4	20.2	0.81	5.80	+15	72–66	1212	36.4	80			
	3:25	10.4	12.5	0.83	3.61	−28	46	720	35.6	65			Shock
	3:45	7.1	9.8	0.73	2.74	−46	22	377	35.7	60			

Experiment XLIV, 5/17/19, 2800 grams, ♂	12:20	19.2	24.6	0.78	7.00		26	3857	37.4	120	11:25		Bled 9 cc. at 12:00 and blood replaced
	1:25	20.9	24.9	0.84	7.21		30	3861	37.4	100			
	1:45	22.7	26.4	0.86	7.67		32	4273	37.5	90			Legs smashed at 2:03
	2:45	23.2	23.7	0.98	7.09		29	6431	36.5	75			Severe shock since 3:00
	3:33	14.1	16.7	0.85	4.82	−34	34–30	4590	37.4	65–60			
Experiment LVI, 12/5/19, 4300 grams, ♂	11:41	23.2	32.0	0.73	8.98			959	38.7	126	10:15		12:45, Bled and transfused. 12:50, Leg smashed
	12:02	24.9	32.0	0.78	9.06			856	38.3	140-132			
	3:19	31.4	30.3	1.03	9.06	0		3295	38.3	75–70			Extreme hyperpnea
	3:36	24.5	24.3	1.01	7.26	−20		3628	38.3	64–59			Extreme hyperpnea

TABLE 5

Respiratory metabolism; shock and transfusions

EXPERIMENT NUMBER, WEIGHT, DATE	TIME OF PERIOD	RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY RATE PER MINUTE	RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	TIME OF ANESTHETIZATION	REMARKS
		CO₂	O₂		Calories	Variation						
		cc.	cc.			per cent		cc.	°C.	mm. Hg.		
Experiment XLI, 5/8/19, 3200 grams, ♂	11:50	15.9	23.1	0.69	6.47		*	599	36.3	145	10:50	Respiration rapid-irregular
	12:15	18.6	25.7	0.72	7.21		114–78	956	36.1	110		Respiration rapid and shallow. 12:55, Bled 10 cc.
	2:55	20.9	26.2	0.80	7.50	+7	78–86	1186	36.3	95		1:10–15, Both legs smashed
	3:30	12.2	18.0	0.68	5.04	−28	50–48	775	35.6	50–63		3:28, 4 cc. blood from right femoral vein. Shock developed at 3:30
	3:45	15.0	20.3	0.74	5.73	−18	41	1365	36.2	67		
	4:30	13.3	16.1	0.83	4.64	−33	27	797	35.4	105		Transfused with 20 cc. blood and 15 cc. mammalian Ringer 4:00. Temperature low. Traube-Herring waves
	4:45	12.9	19.3	0.67	5.40	−23	38–34	727	35.3	80–95		
Experiment XLVII, 7/17/19, 2600 grams, ♀	1:22	17.2	22.0	0.78	6.27		51	571	37.1	92	11:00	Legs smashed at 2:40
	2:20	14.6	21.6	0.67	6.07		63	660	36.7	105		No shock yet
	3:43	14.8	21.6	0.69	6.06	−2	64	692	36.3	82		Shock developing rapidly
	4:05	16.1	17.7	0.91	5.20	−16	83	967	36.4	75–65		
	4:17	12.3	15.7	0.78	4.47	−28	72–176	944	36.2	50–70		
	4:48	13.5	17.7	0.75	4.98	−19	40	493	35.2	95–105		4:25–40, 70 cc. blood transfused. Low temperature unexplained except by transfusion
	5:19	12.7	21.0	0.61	5.88	−5	46	476	36.3	110		
	5:38	14.1	19.7	0.72	5.53	−10	42	482	36.5	105		

Experiment XLVIII, 7/22/19. 2700 grams, ♂	11:45	11.8	18.0	0.66	5.04		37	366	36.3	108	10:20	Normal
	12:12	13.7	18.4	0.74	5.18		36	363	36.2	95		
	12:35	14.8	18.1	0.82	5.20		35	391	36.0	105		
	2:25	11.4	14.5	0.79	4.13	−20	27	395	36.3	60		Onset of true shock level
	2:49	11.8	13.9	0.85	4.03	−22	26–64	550	36.3	65		Respiration increasing rapidly
	3:30	17.2	22.3	0.77	6.34	+23	30	424	36.2	130		3:03–15, 65 cc. blood transfused
	4:02						40	426	36.2	115		Analyses discarded
	4:35	12.7	18.6	0.68	5.23	+2	40	524	36.2	100–115		
Experiment LIII, 11/24/19, 4400 grams, ♂	11:40	23.4	31.8	0.73	8.97			941	38.4	112	10:50	
	12:23	21.7	30.8	0.71	8.63		56	1069	38.3	110		12:55–1:20, Bled, transfused and legs smashed
	2:50	28.1	36.1	0.78	10.28	+17	+	2866	38.3	66		
	3:00	26.8	36.6	0.75	10.35	+18	†	3330	38.4	62		Shock
	3:24	22.2	31.3	0.71	8.77	0	39	901	38.6	116		3:08–17, Bled 15 cc. Transfused 101 cc.
	3:35	20.1	30.8	0.65	8.63	−2	36	927	38.5	124–136		
Experiment LII A, 11/4/19, 4200 grams	11:41	18.6	26.5	0.70	7.43		18	447	36.8	130	10:35	
	12:07											
	12:27	17.7	21.4	0.83	6.17	−17		441	36.8	98		2:00–07, Left leg smashed
	2:50	16.1	19.6	0.82	5.65	−24	16	411	36.9	80		2:50, Cat going into shock
	3:03	17.5	19.3	0.91	5.67	−24	14	463	36.8	74		Cat in shock
	4:01	17.9	20.4	0.88	5.95	−20		327	36.4	100		3:45, 67 cc. blood transfused after bleeding 20 cc.
	4:27	16.1	21.8	0.74	6.15	−17		517	36.6	118		Cat has recovered from shock

* Irregular.

† Intense dyspnea.

TABLE 6

Respiratory metabolism in shock; pericardial pressure

EXPERIMENT NUMBER, WEIGHT, DATE	TIME OF PERIOD	RESPIRATORY EXCHANGE PER MINUTE		RESPIRATORY QUOTIENT	CALCULATED CALORIES PER HOUR		RESPIRATORY RATE PER MINUTE	RESPIRATORY VOLUME PER MINUTE	RECTAL TEMPERATURE	BLOOD PRESSURE	TIME OF ANESTHETIZATION	BLOOD CO_2 COMBINING POWER	REMARKS
		CO_2	O_2		Calories	Variation							
		cc.	*cc.*			*per cent*		*cc.*	*°C.*	*mm. Hg.*			
Experiment III, 10/5/18, 2900 grams, ♀	10:48	12.5	17.0	0.74	4.77		25	406	34.5	145–150	9:15		
	12:11	10.5	11.0	0.96	3.30	−31	varied		35.4	60			12:08, Blood pressure reduced by pericardial pressure
	2:12	10.2	12.5	0.81	3.60	−25	32	527	34.0	60			
Experiment VI, 10/11/18, 7000 grams, ♀ Dog	10:36	57.7	65.8	0.88	19.21		43	2190	34.0	120		50	11:10, Blood pressure reduced by pericardial tension
	11:13	35.8	38.9	0.92	11.48	−41	46	2060	34.5	60		43	
	12:24	41.7	41.2	1.01	12.30	−36	52	2710	34.5	60		37	
	2:23	33.6	30.6	1.10	9.15	−52	48	2950	32.7	60		34	Temperature of dog low
Experiment VII, 10/12/18, 12,930 grams, ♀ Dog	11:07	58.4	77.5	0.77	21.91		46	2280	35.2	120		47	
	11:46	57.8	70.1	0.82	20.19	−8	68	3560	35.2	100		43	Blood pressure reduced gradually by increasing pericardial tension
	12:27	47.8	51.5	0.93	15.22	−31	51	3300	34.8	80		36	
	12:42	38.1	40.9	0.93	12.11	−45	45	3080	34.3	60		30	
	1:20	44.1	45.1	0.98	13.50	−38	33	2450	32.3	80		32	Very low temperature
Experiment XLV, 7/2/19, 2800 grams, ♂	1:06	17.2	24.4	0.71	6.84		64	632	37.0	95	10:30		Variation of 6 per cent. Average taken
	1:40	16.1	23.0	0.70	6.45		62	644	37.4	90			
	2:05	13.2	17.1	0.77	4.86	−27	59	516	37.5	45–55			Taken immediately after increased pericardial pressure 2:46, Cat died

Experiment XLIX, 7/25/19, 2500 grams, ♂	3:34	8.6	13.8	0.62	3.89		43	252	36.2	90	11:15	Cannula in pericardium
	3:58	10.3	13.9	0.74	3.90		46	258	36.4	93		4:24, Intrapericardial pressure increased by gum-salt solution
	4:25	9.5	11.7	0.81	3.37	−13	38	285	36.5	55		
	4:44	9.0	10.6	0.85	3.07	−21	40	278	36.6	58		
	5:03	8.8	10.6	0.83	3.06	−21	42	296	36.8	59		Cat killed
Experiment LIV, 12/11/19, 3300 grams, ♂	12:00	16.3	23.8	0.69	6.67			478	38.0	142–116	10:20	A control experiment to show recovery after a low blood pressure
	12:19	17.8	24.3	0.73	6.82			544	38.0	108		
	2:14	17.2	23.2	0.74	6.55	−3	32	516	38.3	112		At 1:11, Blood pressure reduced to 60. At 2:10, pericardial pressure removed
	2:37	16.4	21.4	0 77	6.06	−10		549	38.3	110		

extremely complicating factors which may be influencing them. However, the oxygen absorption from the lungs must represent the amount of oxygen available for metabolic uses in the tissues, inasmuch as the blood leaves the heart normally saturated with oxygen (16). That this absorbed oxygen represents the amount used by the body seems highly probable, as otherwise there would be an accumulation of oxygen in the tissues, a condition which seems decidedly unlikely. In all these observations, therefore, the oxygen absorption has been used as the basis of comparison, and the CO_2 assumes a relatively unimportant rôle through its influence on the respiratory quotient.

The reduced metabolism affords an explanation for the marked reduction of body temperature in shock, which may go as low as 87.8°, or even 76.1° in cervical spine lesions, according to Weil (18) and to Volkmann (17). This investigation, however, does not indicate that the reduction is usually a forerunner of the onset of shock, or that it is a causative factor in the production. In fact, in two experiments the metabolism just before the onset of shock was slightly elevated above the normal level.

The volume of respiration per minute has likewise been studied. The average ventilation per minute of the control observations was 557 cc. in twenty-one experiments; the average after crushing the muscles and before the onset of a shock blood pressure level was 860 cc. in ten experiments, (a variation of +54 per cent from the control observations), and after the onset of shock it was 635 cc. in thirteen experiments. This variation is hardly enough to account for the onset of shock, in these muscle trauma experiments, by the acapnia theory advanced by Henderson and Haggard (19), (20). Besides, rapid breathing with a higher ventilation rate per minute than in shock has been repeatedly seen under urethane anesthesia without the onset of spontaneous shock. These data also show that the fall of the metabolic rate was not due to changes in either the volume or exertion involved in respiration.

With the metabolism so much reduced by shock, it naturally became of interest to know the effect of transfusion of a sufficient amount of blood to bring about recovery. Table 5 shows five such experiments in three of which the metabolism returned to normal limits, while in experiment XLI the metabolism remained low. In experiment XLVIII, the figures for the first period after transfusion were above the normal determinations. Experiment LIV in table 6 shows the effect of reducing the pressure to shock level by pericardial pressure.

Periods III and IV were taken directly after releasing the pressure, and showed a normal rate. It therefore seems that making the circulation adequate causes the low metabolism of shock to disappear promptly.

CONCLUSIONS

1. Ethyl carbamate (urethane) is a satisfactory anesthetic for the study of gaseous metabolism in animals over short periods of time.
2. Experimental traumatic shock causes a marked fall in the rate of basal metabolism to 70 per cent of the original level. The degree of fall is dependent upon the severity of the shock produced.
3. A similar fall of the metabolic rate may be rapidly accomplished by interfering with the circulation by increased pericardial pressure.
4. The effect of hemorrhage is not constant. It may temporarily lower, or have no immediate effect on the metabolic rate.
5. Recovery from shock after blood transfusion is usually associated with a prompt return of the metabolic rate to a normal level.

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STUDIES IN EXPERIMENTAL TRAUMATIC SHOCK

II. The Oxygen Content of the Blood

JOSEPH C. AUB and T. DONALD CUNNINGHAM

From the Laboratory of Physiology in the Harvard Medical School and the Medical Clinic of the Peter Bent Brigham Hospital[1]

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In studying the basal metabolism of experimental traumatic shock it became clear that the reduction of the calories utilized was dependent upon some undetermined factor. This was suggested by the fact that the fall in metabolic rate did not always coincide with the fall in blood pressure. Other observers have noted that there were marked changes in the circulation before a shock level of blood pressure was approached. Gesell (1) showed a slowing of blood flow through the salivary gland before a fall in pressure had developed. Yandell Henderson, (2) while working with shock induced by intestinal trauma in dogs, found a markedly decreased O_2 content in the venous blood, which he thought followed the failure of the venopressor mechanism and demonstrated a true anoxemia.

These observations suggested that the determination of the oxygen of the arterial and venous blood, as well as the blood flow during the development and recovery from traumatic shock, might give evidence as to the cause of the fall of metabolism (3).

Method. The animals used were cats, and the methods used for inducing traumatic shock and determining metabolism were similar in all respects to those previously described (3). The values for blood oxygen were obtained by the methods of Van Slyke (4).

The blood was withdrawn in two ways: *a*, by inserting a needle in a branch of the femoral artery and vein, and so entering the larger vessels without causing any stasis; *b*, the more satisfactory way, by inserting thin catheters down the right carotid artery and right superficial jugular vein until they reached the heart. The blood was collected in glass syringes, under paraffine oil, and put into tubes under oil (5).

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[1] This is study no. X of a series on the physiology and pathology of the blood from the Harvard Medical School and allied hospitals.

By this method blood could easily be obtained without exposure to air. In order not to disturb the blood volume in the experimental animal, the amount removed for analysis was promptly replaced by an equal amount of citrated cat's blood. Thus fairly large samples of arterial and venous blood could be obtained without permanently affecting the blood pressure or blood volume.

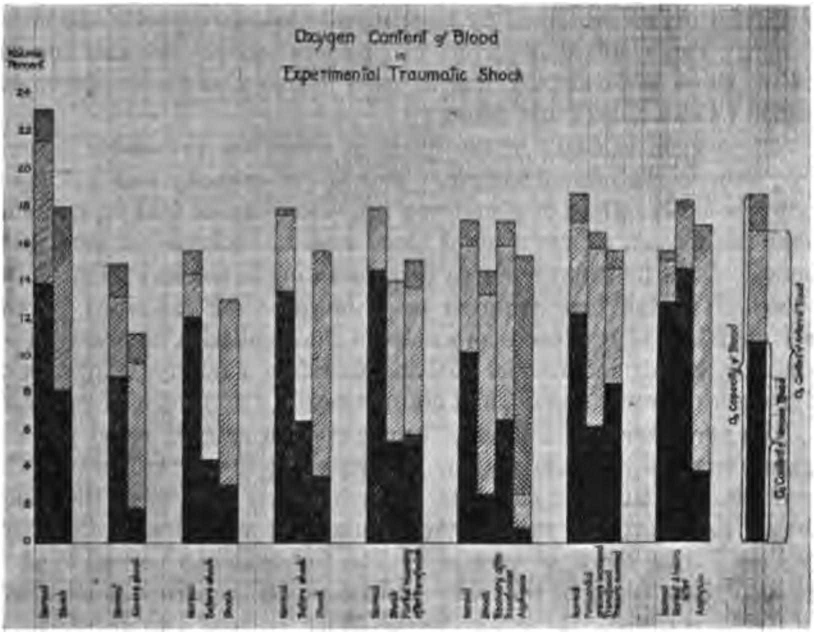

Fig. 1. Normal = Animal under urethane anesthesia.
Before shock = After muscle trauma, but before a true shock level of blood pressure had been established.
Asphyxia = Clamping off trachea completely for 4 or 5 minutes.

After shock had been established for about 30 minutes, as shown by a fall of blood pressure to 60–70 mm. Hg., the blood samples were taken and were immediately followed by a large transfusion of blood, as much as 100 cc. being given in an attempt to relieve the shock. In one case this accomplished a permanent recovery of blood pressure to its original level. In cases where a few minutes' delay followed the taking of the blood samples from the shocked animals, only slight recovery of blood pressure followed the transfusion.

Discussion. Figure 1 shows graphically the more important changes which occur in the oxygen of the blood in traumatic shock. The most striking effect is seen in the oxygen content of the venous blood, which falls very markedly in shock—not only in actual content of oxygen but in percentage of saturation.[2] This is well shown in all the experiments in table 2, but possibly most notably in experiments LIII and LV, and is also present in a control experiment LIV, table 1, where a low pressure was obtained by mechanically interfering with the circulation. The changes observed in profound shock are also present as the shock is developing, and to lesser degree after the improvement which follows large transfusions.

The oxygen capacity of the blood in shock has invariably become less than in the normal sample. This fall in capacity does not agree with the observations of Henderson (2), who found in four experiments that the oxygen of the arterial blood rose 1.5 volume per cent after shock. This he interpreted as demonstrating a concentration of the blood. The fall here reported may, however, be explained by the accumulation of red blood corpuscles in the capillaries, as observed by Cannon, Fraser and Hooper (6), and therefore a relative reduction of corpuscles in venous blood, and not necessarily a dilution of the plasma.

The percentage saturation of hemoglobin in arterial blood has not varied markedly in the various conditions of the experiments. The ventilation is at least adequate throughout, so that when the blood leaves the heart and reaches the tissues it is as well saturated with oxygen during shock as normally. The fall from the normal level of oxygen takes place in the venous blood, which confirms Henderson's observations, and this occurs before shock as well as during shock. In experiment LVI, while the blood pressure was, and had been, 104–94 mm. Hg. for 1½ hours after the muscle injury, the oxygen content of the venous blood had fallen from 12.27 to 4.55 volumes per cent. So also in experiment LVII, although the blood pressure was 84 mm. Hg. after the muscle injury, the oxygen content of the venous blood had fallen from 13.68 to 6.64 volumes per cent.

A similar, though less marked, decrease in the oxygen content of the venous blood is seen after recovery by transfusion as seen in experiment LIII, table 2. That this is sufficient to indicate a true asphyxia in the tissues cannot now be proved because the head of oxygen pressure necessary for normal oxidation is not yet definitely known. However, the very small amount of oxygen present (with the attendant low partial pressure) makes a true anoxemia possible.

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[2] The percentage of saturation as used in this paper, represents the oxygen content of venous blood divided by the oxygen capacity of the arterial blood.

It is true, however, that the venous blood may be as low in oxygen in severe anemia as it is in shock, but in this condition the oxygen of the arterial blood is likewise reduced. Morawitz and Rohmer (7) found that in three human cases of very severe anemia, where the oxygen-carrying capacity of the blood was 4.5 per cent or less, the venous blood had an oxygen content as low as 0.67 per cent, and they assumed an increased blood flow to explain the normal metabolism which is still found in these cases. Lundsgaard (8), also studying patients with anemia, found the venous blood contained as low as 1.16 volume per cent of O_2 in a case with an oxygen-carrying capacity of 5.93 per cent. He concludes that the tissues extract oxygen from the blood with equal readiness whether there is a large oxygen reserve in the blood, "or practically no reserve, as in anemia." These cases, however, had very low oxygen capacities, and therefore the low O_2 content of the venous blood meant a less complete dissociation[3] of hemoglobin and O_2 than would a similar figure in a normal blood. It is the amount (percentage) of this dissociation which must influence the partial pressure of the dissolved oxygen, and this latter is the important factor in the migration of oxygen into the tissues. The oxygen content of normal blood may, therefore, be three or four times that of anemic under the same partial pressure of O_2. As a result, under similar conditions, one would expect to find a much larger figure for the total venous content of oxygen in these shock experiments than would be found in anemia, because anemic blood has less hemoglobin. In fact, the *percentage* saturation of the venous blood in these anemia cases, (16 per cent and 20 per cent), is about the same as in the cases of severe shock, in spite of the lower venous *content*.

A more direct control of the value of the oxygen content found in these experiments are the figures obtained in animals after 4 or 5 minutes of complete asphyxia, for here, just before death, the oxygen value of the venous blood was not very much lower than that found in shock. This is of course indirect evidence, for the matter of greatest importance to the tissue is the oxygen content of the arterial blood. Still, the oxygen content of the venous blood must give a satisfactory indication of conditions in the venous end of the capillaries, and in the asphyxia experiments must surely indicate an oxygen content which is entirely inadequate for the use of the tissues.

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[3] This dissociation is approximately represented by the "per cent of saturation" column in tables 1 and 2. It is approximate, because the small amount of oxygen dissolved in the plasma would be about the same in anemic and normal bloods.

The explanation of this marked anoxemia lies most probably in a slowed blood flow which has been demonstrated in shock, and which can be very well demonstrated in these experiments by the method used by Means and Newburgh (9). In brief it is based on the formula:

$$\text{Volume output of heart per min.} = \frac{\text{cc. } O_2 \text{ absorbed through lungs}}{\text{Volume per cent oxygen utilization of blood}}$$

Using this formula we can calculate the blood flow of several of our cases. The results are shown in tables 1 and 2. It is clear that the blood flow becomes markedly slowed before the onset of a shock level of blood pressure, and that this slowing precedes the fall in metabolism, demonstrated in paper I.[4] This is demonstrated in experiments LVI and LVII. It may therefore be assumed that the fall in metabolism is a secondary manifestation of the decreased blood flow, and of the markedly reduced oxygen content of the venous blood, and is probably due to a true anoxemia. This suggestion is further borne out by the similar findings following increased pericardial tension (exper. LIV), for this must suddenly decrease the rapidity of blood flow. There is a much increased oxygen utilization in the blood and there is also a very prompt fall in the level of the basal metabolism (3), although the only disturbing factor is in the circulation, and no toxic effect from tissue injury can be involved. Verzar (10) has also shown that when perfused muscles are given inadequate oxygen supply the height of their metabolism falls.

Under these conditions we may add another factor to the vicious cycles described by Cannon (11). Krogh (12) has shown that as an oxygen want develops in contracting muscles, many empty capillaries fill with blood, a change which reduces the distance necessary for the diffusion of gases into the tissues. Thus, as anoxemia develops, the capillary bed would increase in volume. This would further decrease the already slowed blood flow, and the slower the flow the greater would probably become the oxygen consumption per cubic centimeter of blood, and hence the decrease in oxygen of venous blood.

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[4] Experiment LIII does not show so striking a drop as the other two. It was abnormal, however, in being the only observation which showed a rise in metabolism during shock, instead of a fall. This was probably due to the intense dyspnea.

TABLE 1

Normal controls

NUMBER, WEIGHT, DATE	TIME OF SAMPLE	O_2 OF ARTERIAL BLOOD		O_2 OF VENOUS BLOOD			HEMOGLOBIN CALCULATED	CO_2 COMBINING POWER	BLOOD PRESSURE	METABOLIC RATE O_2 ABSORBED PER MINUTE	BLOOD FLOW PER MINUTE	REMARKS
		Capacity	Content	Content	Consumption	Saturation						
		vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	*per cent*	*vol. per cent*	*mm. Hg.*	*cc.*		
LIX	11:57	15.93	15.38	13.12	2.26	82.4	86.1		92	24.9	1103	Transfused 30 cc. after bleeding
3.5 kgm.	4:07	18.68	18.35	14.93	3.42	79.9	100.7		110	21.5*	630	Transfused 45 cc.
12/12/19	4:24	17.33		3.89		22.4			50			After 4 minutes asphyxia
LXIX	12:55	15.7	16.41	11.80	4.61	75.2	83.6		110	21.6	469	
2.7 kgm.	4:35	15.0	16.38	9.50	6.88	63.3	82.4		108	19.5	284	
5/27/20												
LVIII	2:10	20.92	19.67	14.30	5.37	68.4	113.0		100	20.5	383	
2.9 kgm.	4:35	21.87	17.88	13.47	4.41	61.6	117.0		120	23.8	539	
4/27/20												
LXII	2:45	17.5?	18.0	9.90	8.1	55.0	89.0		100	23.2	283	
2.6 kgm.	5:00	18.4	18.0	9.10	8.9	49.5	93.0		155	22.9	253	
5/14/20												
LIV	12:47	18.96	17.45	12.50	4.95	65.9	102.0	57.4	112	24.0	485	Normal
3.3 kgm.	1:50	16.9	16.07	6.37	9.70	37.7	91.0	46.8	60			Blood pressure reduced by pericardial pressure for 45 minutes
12/1/19	2:50	15.92	14.92	8.69	6.23	54.6	86.0	47.7	105	22.3	358	Normal for 35 minutes

* Five and one-half hours after anesthesia.

TABLE 2

Experimental traumatic shock

NUMBER, WEIGHT, DATE	TIME OF SAMPLE	O_2 OF ARTERIAL BLOOD		O_2 OF VENOUS BLOOD			HEMOGLOBIN CALCULATED	CO_2 COMBINING POWER	BLOOD PRESSURE	METABOLIC RATE O_2 ABSORBED PER MINUTE	BLOOD FLOW PER MINUTE	REMARKS
		Capacity	Content	Content	Consumption	Saturation						
		vol. per cent	vol. per cent	vol. per cent	vol. per cent	per cent	per cent	vol. per cent	mm. Hg.	cc.		
LI	2:35	17.3	18.1	14.8	3.3	85.6	95.0	36.0	124			Normal
4.1 kgm.	4:20	13.9	14.2	5.5	8.7	39.6	75.4	34.1	56			Mild shock for 1 hour
10/22/19	5:00	15.3	13.8	5.9	7.9	38.5	82.7	40.6	81			After transfusion 43 cc. blood
LII	3:30	23.3	21.6	14.0	7.6	60.2	120.3	40.2	143			Normal
4.9 kgm.	6:05	18.1	14.2	8.3	9.8*	45.9	98.0	37.1	68			Shock for 1 hour
10/29/19												
LIII 4.4 kgm. 11/24/19	12:55	17.46	16.16	10.32	5.84	59.1	94.5	40.4	120	31.3	536	Normal
	3:08	14.76	13.46	2.69	10.77	18.2	79.8	33.8	62	36.4	337	Shock for 30 minutes
	4:29	17.46	16.17	6.73	9.44	38.5	94.5	40.4	125	31.0	328	Recovery after transfusion of 100 cc. blood
	4:34	15.59	2.69	0.89	1.8	5.7	89.6	43.3	70			Asphyxia 3 minutes by clamping trachea
LV	12:55	15.02	13.30	8.99	4.31	59.9	81.1	41.9	80			Normal. Transfused 27 cc.
3.1 kgm.	2:35	11.32	9.73	1.87	7.86	16.5	61.1	25.6	44			Very severe shock for 30 minutes
12/4/19												
LVI	12:35	15.77	14.54	12.27	2.27	77.8	85.1	48.2	132	32.0	1390	Normal
4.3 kgm.	3:02			4.55	7.70†				94	30.3	400†	Before shock developed
12/5/19	4:00	13.16	Clotted	3.18	9.98†	24.2	69.4	45.4	52	24.3	272	Severe shock
LVII	12:47	18.09	17.78	13.68	4.10	75.6	97.8		120	31.0	756	Normal
4.6 kgm.	1:58			6.64					84	27.5	308†	Before shock developed
12/10/19	3:25	15.81		3.65	12.16†	23.1	85.5	39.3	70	24.5	206	Mild shock

* Animal stopped breathing just before taking sample.

† Capacity substituted for oxygen content. These figures therefore approximate.

CONCLUSIONS

1. There is a markedly diminished oxygen content of the venous blood in experimental traumatic shock. This change occurs before the blood pressure falls to a shock level, and is still present after apparent recovery from shock.

2. The blood flow is also greatly decreased in the development of, during and after shock.

3. The resulting anoxemia of the tissues may be the cause of the decreased metabolism.

4. The sequence of these events in traumatic shock is discussed.

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STUDIES IN EXPERIMENTAL TRAUMATIC SHOCK

III. Chemical Changes in the Blood

JOSEPH C. AUB and HSIEN WU

From the Laboratories of Physiology and Biochemistry in Harvard Medical School

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In experimental traumatic shock there is a marked decrease in the rate of blood flow and of general metabolism. Decreased blood flow and blood pressure result in a diminished secretion by the kidney (1). It is of interest to know what are the effects of such acute abnormal conditions upon the chemical constituents of blood, especially as a chemical cause of shock is now being seriously considered.

Some work in this field has been done by French investigators. Duval and Grigaut (2) studied the non-protein nitrogen of the blood in shock and concluded from their results that in the war-wounded there was an increase in the non-protein nitrogen of the blood, which started promptly after the wounding, was at its height during the second day and then gradually returned to normal. This increase was slight in unshocked cases, whether the wounds were infected or not. The retention differed from that found in nephritis in that the nitrogen increase in blood occurred not markedly in the urea portion but in the remainder of the non-protein nitrogen.

Whipple and his collaborators (3) studied the blood in the intoxication following intestinal obstruction, and after injection of the toxic proteoses which developed in obstructed intestines or in closed intestinal loops. The response to these injections was one which in many ways resembled traumatic shock,—with a fall in temperature and blood pressure. The injection was followed by a large increase (40 per cent or more) in the non-protein nitrogen of the blood, but this increase was found chiefly in the blood urea nitrogen, although the amino- and peptid-nitrogen also showed slight increases.

It is thus seen that the conclusions of the French and of the American investigators are somewhat contradictory, though it may be conceded that the response obtained by proteose injection is not a true shock.

At any rate, neither of these investigators have found any chemical change in blood other than rise in non-protein nitrogen which may be regarded as characteristic of traumatic shock.

The method of analysis used in our experiments was that of Folin and Wu (4). The urea was always determined by means of urease and aeration, as hydrolysis with the autoclave would also decompose the urethane used as an anesthetic. The figures obtained by the latter method were 10 to 15 mgm. too high, and it may therefore be concluded that the blood in our experiments contained about this amount of urethane N per 100 cc. The urethane contributed but little to the non-protein nitrogen as actually determined. It is so volatile that most of it is expelled in the course of the digestion. Experiments with a pure urethane solution containing 10 to 15 mgm. N per liter (i.e., as much urethane N as the blood filtrate might contain) have shown that only 3 to 4 mgm. N were fixed by the acid digestion mixture. The values of the total non-protein N obtained are, therefore, only 3 to 4 mgm. higher than the actual total non-protein nitrogen minus urethane nitrogen.

The creatin N represents as usual the difference between the total creatinine and the preformed creatinine multiplied by 0.37. In the first few experiments both the total creatinine and the preformed creatinine were determined, but as the latter showed no appreciable variation during anesthesia,—averaging 2 mgm. creatinine per 100 cc. blood, in control as well as in shocked animals[1]—its separate determination was discontinued in later experiments in order to economize the blood filtrate. Its average value was used for the calculation of the creatin.

In tables 1 and 2 are shown the results of our experiments. It is clear that in the control experiments there was no marked rise in any of the chemical constituents studied. This is true even in the experiments where the blood pressure and the blood flow were reduced by mechanical means but without muscle trauma (exper. LIV). In the animals in shock, however, there was usually a marked increase of all the constituents which we determined, over what was present before

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[1] In a control experiment, 1 hour and 10 minutes after anesthesia the blood contained 5.2 mgm. total creatinine and 2.1 preformed creatinine per 100 cc. blood. Five hours later the total creatinine was again 5.2 mgm. and the preformed creatinine was 1.8 mgm. In another experiment (2 hours after traumatization) the blood contained 15 mgm. total creatinine and only 2.3 mgm. preformed creatinine.

TABLE 1

Control experiments; blood analyses in untraumatized animals

EXPERIMENT NUMBER, DATE	TIME OF BLOOD SAMPLE	BLOOD PRESSURE	TIME OF BLOOD PRESSURE BELOW 80 MM. Hg.	BLOOD ANALYSES, MILLIGRAMS PER 100 CC. OF BLOOD					BASAL METABOLISM		REMARKS
				Total non-protein nitrogen	Urea		Creatin nitrogen	Sugar	Time	Calories per hour	
					Nitrogen	Per cent					
		mm. Hg.									
XXVII 3/31/19	12:49	158		48	26	54	1.2	282	— 1:28	— 7.34	Normal control
	5:10	141		47	30	64	1.2	280	4:30 5:30	7.19 6.84	
7/15/19	10:44	110		52	29	56	1.9	206	11:00 11:22	4.10 4.19	Normal control
	5:30	94		55	32	58	1.8	286	4:19 5:11	3.43 3.20	
11/18/19	11:34	114		52	28	54	1.4	276			Blood pressure reduced by intrapericardial pressure. No muscle trauma
	1:10	60	90	58	29	50	1.7	328			
LIV 12/1/19	12:40	114		48	26	54	1.2	310	12:15	6.75*	Blood pressure reduced by intrapericardial pressure
	1:45 2:00	65	40	47	28	60	1.2	324			Pericardial pressure removed. Cat transfused. Good recovery of original blood pressure
	2:50	116		50	28	56	1.1	282	2:25	6.31*	

11/19/19	11:16	146		58	29	50	1.3	320			Under ether anesthesia
	12:35	70	20	66	32	49	1.3	334			Under urethane for 1 hour 15 minutes
XXXIV 4/19/19	11:30	106		48	28	58	1.5	250	11:11 11:42	5.73 5.16	Hemorrhage alone. Blood pressure markedly reduced by taking blood sample. No trauma
	2:25	108		43	29	67	1.6	276	1.51 2.35	5.69 5.32	

* Average.

TABLE 2

Blood analyses after muscle trauma

EXPERIMENT NUMBER, DATE	TIME OF BLOOD SAMPLE	BLOOD PRESSURE	TIME OF BLOOD PRESSURE BELOW 80 MM. HG.	BLOOD ANALYSES, MILLIGRAMS PER 100 CC. OF BLOOD					BASAL METABOLISM		REMARKS
				Total non-protein nitrogen	Urea		Creatin nitrogen	Sugar	Time	Calories per hour	
					Nitrogen	Per cent					
		mm. Hg.									
6/9/19	11:10	155		60	42	70	2.0	286			Cat severely traumatized but no true shock. Blood pressure never below 83
	1:40	93		53	34	64	2.2	400			
6/19/19	11:17	126		53	39	74	1.8	296			After trauma to both legs blood taken as cat seemed about to go into shock
	1:00	80		65	42	65	1.3	334			
6/14/19	11:26	158		55	32	58	2.0	222			Muscles severely traumatized. Marked drop in pressure but no true shock level
	2:40	85		57	37	65	3.0	258			
10/21/19	2:34	110		60	35	58	2.4	320			Severe muscle trauma at 3:00 with prompt drop of blood pressure below 80. Transfusion at 4:26 with only partial recovery (46 cc. blood)
	4:15	60	70	70	40	57	2.8	440			
	4:50	82		71	40	56	2.8	440			
XXXIII 4/15/19	11:15	115		55	35	64	1.3	196	10:50 11:37	7.10 5.55	Legs smashed at 12:08. Very mild shock and no marked drop in respiratory metabolism
	2:48	80	140	64	43	67	3.5	400	2:42	6.15	
XXXV 4/22/19	12:40	84		53	25	47	1.5	222	12:27 12:51	5.28 4.84	Marked reduction of blood pressure from hemorrhage alone. Legs smashed at 2:12 with prompt shock developing
	3:58	42	280	77	42	55	6.1	364	3:53	3.76	

XL 5/6/19	1:00	100	Few mins.	53	25	47	1.9	242			Very sudden onset of shock after severe resmashing of muscles with prompt death
	3:20	60		52	28	54	3.7	392			
XXXVII 4/28/19	1:55	126		50	28	56	2.2	250	1:36 2:06	8.06 8.16	Legs smashed at 2:25 and 3:25. Severe shock
	6:20	52	80	70	42	60	5.7	400	6:02	5.35	
XXXVIII 5/1/19	1:00	80–100		51	35	67	2.2	296	12:24 1:17	5.08 4.63	Both legs smashed at 1:35 and1:55. Severe shock
	3:55	62	70	74	49	66	6.0	400	3:55	2.74	
XXXIX 5/5/19	12:22	104		47	25	53	1.8	306	12:02 12:51	6.30 6.07	1:05. Both legs smashed. Mild shock
	2:40	74	90	59	33	48	4.5	550	1:59	5.61	
XLI 5/8/19	12:55	116		46	20	43	1.5	188	12:39	7.30	1:00. Both legs smashed. Shock developed on removing blood for analysis. Transfusion at 4:00 with 20 cc. blood. Sample IV is blood from femoral vein leaving traumatized muscle. Sample V is taken from carotid artery at same time
	3:28	84		50	23	46	4.2	250	3:00 3:35	7.50 5.04	
	Blood used for transfusion			51	29	57	2.2	276			
	5:00			49			4.8				
	5:00	80		50	24	48	3.7	333	4:49	5.40	
6/25/19	11:10	125		50	28	56	2.2	296			Blood CO_2 combining power 36.5 at 12:06. Rapidly developing very severe shock. After muscle trauma blood CO_2 combining power 22.3 volumes per cent
	2:15	70	20	57	32	56	3.2	400			

trauma. This increase was not marked in traumatized animals which did not go into shock,—and, in fact, the rise tended to run parallel with the severity of the shock.

The most significant result of the blood analysis is that in connection with the creatin. In control animals the creatin content of blood remained practically unchanged during the five hours of the experiment. In animals which were traumatized but had not gone into shock the blood creatin showed an unmistakable increase. When shock developed, the creatin figure rose frequently to three times the normal. The source of the increased creatin in blood is the injured muscle. This is not only in accord with the known facts but is also shown in experiment XLI. The blood from the femoral vein contained distinctly more creatin than did the carotid blood. According to the views of Folin and Denis (5), creatin does not exist as such in the intact muscle and it is a post-mortem product set free by the dying tissue. While these investigators based their view on indirect though convincing data, the results of our experiments seem to afford direct evidence.

The parallelism between blood creatin and the severity of the shock does not of course indicate that creatin itself is responsible for this condition. Creatin is innocuous even in large doses. Simultaneously with the liberation of creatin from the autolyzing muscle there is probably formed a large number of other nitrogenous substances—possibly histamine and the like, and to these substances may possibly be ascribed the cause of shock. The increased blood creatin is merely an index of the extent of the necrosis which the injured muscle has undergone. Since creatin is derived solely from the injured muscle, the results of our experiment seem to afford suggestive chemical evidence in support of the view already prevalent that the shock is produced by some substance coming from the injured muscle (6), (7).

Although an increase in the total non-protein nitrogen always attended the development of shock, the increase was slight except in cases where the shock was profound. This relatively slight increase of total nitrogen is an excellent check on the increase of creatin, for it shows that the accumulation is due to increased production and not simply to kidney inefficiency, which would probably cause a parallel rise of all constituents. We are inclined not to attach much significance to the variation of the total nitrogen (and the percentage of urea N) on account of the complication by the urethane, but our results agree rather well with those of Whipple and collaborators (3), and it may be concluded that albumoses, as such, could not have played any important rôle in the development of shock.

Some work has been done on the blood sugar content in traumatic shock. Cannon (8) reported that there was surely a normal amount if not a slightly increased blood sugar in wound shock. Fabre, Wertheimer and Clogne (9), however, report a reduced blood sugar content in shock. In our control experiments the blood sugar, while always high on account of anesthesia, did not rise greatly in the second sample. In the traumatized cases, however, there was nearly always definite and marked rise in the sugar content of the blood. Too much stress may not be laid upon the extent of the rise, however, because of the urethane. That this anesthetic may affect the carbohydrate metabolism was suggested by the work of Underhill (10), who showed that adrenalin glycosuria was more readily obtained when urethane was the anesthetic than otherwise. The rise of sugar values which we have found, however, is very striking and difficult to explain. Three possibilities suggest themselves: *a*, It may be the hyperglycemia associated with activity of the sympathetic nervous system; *b*, The reduced total metabolism might be used to explain an accumulation in the blood,—but the respiratory quotients in traumatic shock (11) suggest that at least a normal proportion of carbohydrate is being metabolized; *c*, finally one may look to the liver for an explanation. Several French observers have ascribed shock phenomena to a liver insufficiency, and one might assume that this rise in blood sugar is due to a loss from the glycogen commonly stored there. This might be a direct result of the toxic alterations which may occur in the liver (12).

CONCLUSIONS

1. Animals with marked muscle trauma but without true shock showed only slight changes in total non-protein nitrogen, urea, creatin and sugar in the blood. These constituents, especially the creatin and the sugar, rose markedly as shock developed. In control animals the determined constituents showed no appreciable change.
2. The marked rise in creatin is direct evidence of the presence in the blood of products of muscle necrosis, and is therefore suggestive evidence for the theory of the chemical cause of traumatic shock.
3. The cause of the rise in blood sugar is briefly discussed.

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EXPERIMENTAL STUDIES IN DIABETES

SERIES II. THE INTERNAL PANCREATIC FUNCTION IN RELATION TO BODY MASS AND METABOLISM

7. *The Influence of Cold*

FREDERICK M. ALLEN

From the Hospital of The Rockefeller Institute for Medical Research, New York

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According to some earlier literature (1), cold causes hyperglycemia and glycosuria in both warm- and cold-blooded animals, and increases the glycosuria of diabetic dogs. The use of cold and shivering to drive out glycogen from phlorizinized animals was familiarized by Lusk. A single mild test of cold environment in a human patient gave a negative result (2).

A few words may be devoted to the theory of the subject. In a totally phlorizinized dog, it is obvious that a release of carbohydrate which cannot be utilized will result in a temporary rise of glycosuria and of the D : N ratio. A similar mobilization of stored carbohydrate, together with a possible diuretic action of cold, might produce a similar result in a totally depancreatized animal. The extra heat required to maintain body temperature would supposedly be furnished by fat, and there is no reason to expect any increased output of sugar derived from protein, either in the sense of any appreciable increase of protein decomposition or any genuine alteration of the D : N ratio. In a partially depancreatized animal, the increased carbohydrate mobilization or increased total metabolism may impose an additional burden upon the pancreas remnant, and any lasting increase of glycosuria must be interpreted in the sense of a true aggravation of the diabetes. At the same time complicating factors come into play. If the animal fasts, the extra sugar loss and fat combustion impose a sharper under-nutrition treatment in a cold than in a warm environment. Likewise a diet which is adequate in warmth becomes under-nutrition in the cold. On the other hand an increase of diet to meet the increased requirement complicates the problem still further. Shivering is a form of muscular exercise, and may possibly have a metabolic effect opposed to the effect of cold *per se*.

Normal dogs can live without artificial heat in the ordinary winter weather of New York if merely sheltered, but their metabolism evidently is considerably higher than that of dogs kept in warm rooms. The first experiments were therefore planned to determine whether there is any practical difference in the ease of producing diabetes in dogs kept in outdoor cages in winter and in others kept in a room specially warmed to a high summer temperature. This method was considered better for the purpose than the plan of using severely diabetic dogs and following the variations in their glycosuria, because it was important to distinguish a tendency to the production of mere glycosuria and hyperglycemia (such as cold may excite even in normal animals, which are certainly not diabetic from this cause) from a tendency to the production of actual diabetes. If cold has any diabetogenic action, equivalent to the removal of a small fraction of a gram of pancreatic tissue, this action should be demonstrable in such tests.

Twenty-five dogs were used for this investigation, with removal of such portions of pancreas as were known to produce a close approach to diabetes.[1] These experiments included fasting and fixed diets, also single and repeated operations, the latter as usual removing successive bits of tissue till diabetes resulted. Comparisons were made between dogs kept in the warmth and others kept in the cold, and also in the same animals by sudden changes from one environment to the other. The animals chosen ranged from small short-haired dogs which were highly sensitive to the cold and might sometimes be unduly depressed by it, to large woolly dogs which scarcely shivered in the winter weather. The general technic of such experiments is sufficiently clear from the preceding papers, so that brevity may be served by omitting protocols. In two instances the change from warmth to cold seemed responsible for a definite but transitory glycosuria. Otherwise the results were negative, and the conclusion was established positively that there is no demonstrable difference in the amount of pancreatic tissue that must be removed to produce diabetes in dogs in warm or cold environment. An effect of cold upon the islands of Langerhans was also not observable.

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[1] All operations were performed under ether anesthesia.

Although cold has no diabetogenic influence whatever in the sense of this test, it thus merely conforms to the rule that the most powerful functional influences avail little in comparison with the smallest fraction of a gram of healthy pancreas tissue. As agencies which are negative in this respect sometimes appreciably influence the course of an existing diabetes, some experiments concerning hyperglycemia and glycosuria were performed upon dogs with various grades of carbohydrate tolerance. For this purpose the animals' cages were transferred from a comfortably warm room to a refrigerator room kept at approximately freezing temperature. The observations are arranged in a series chiefly according to descending assimilative capacity.

Dog C3–36

TIME	PLASMA SUGAR	REMARKS
	per cent	
August 6		
10:00 a.m.	0.128	At summer temperature
3:40 p.m.	0.116	Immediately after this bleeding, transferred to ice room
5:50 p.m.	0.098	
August 7		
10:00 a.m.	0.113	
4:00 p.m.	0.100	Immediately after this bleeding, transferred from ice room to summer temperature
6:00 p.m.	0.095	
9:25 p.m.	0.105	

The normal dog C3-36, weighing 17 kilos, was placed in the ice room after the bleeding at 3:40 p.m. on August 6, and left there until after the bleeding at 4 p.m. on August 7. The usual diet of bread and soup was fed each evening after the final blood sample was taken. The dog was in excellent condition and powerfully muscled, but very short haired, and shivered continuously in the cold. The plasma sugar seemed to be affected very slightly if at all. At any rate, no elevation by cold was observed.

Dog B2-00, mentioned several times in previous papers, in August, 1915 was close to the verge of diabetes, but had been free from glycosuria on fixed bread diet for several months. Exercise had proved able to modify the blood sugar and the glucose assimilation considerably, and an experiment with cold was therefore performed under similar conditions. The diet was fed each evening after the last blood test. There was no glycosuria. The plasma sugar was slightly lower at the low temperature.

TIME	PLASMA SUGAR	REMARKS
	per cent	
August 5		
11:30 a.m.	0.111	
2:30 p.m.	0.117	At summer temperature
August 6		
9:30 a.m.	0.110	
3:30 p.m.	0.098	Immediately after this bleeding, transferred to ice room
5:45 p.m.	0.085	
August 7		
9:30 a.m.	0.098	
3:30 p.m.	0.107	Immediately after this bleeding, transferred from ice room to summer temperature
6:00 p.m.	0.125	
9:30 p.m.	0.112	

Dog B2-01

PLASMA SUGAR			TIME
September 21, Cold	September 29, Control	October 6, Exercise	
per cent	*per cent*	*per cent*	
0.116	0.106	0.105	Blood before feeding
0.192	0.256	0.147	1 hour after feeding
0.106	0.147	0.148	3 hours after feeding
0.131	0.103		6 hours after feeding

Dog B2-01, likewise near the verge of diabetes, received 56 grams Merck anhydrous glucose (4 grams per kilo) in 30 per cent solution by stomach tube on three days, respectively in the cold, in warmth at rest, and with treadmill running. The control day showed the highest plasma sugar curve, while both cold and exercise seemed to depress it.

Dog B2–02

PLASMA SUGAR			URINE SUGAR		TIME
July 20, Control	September 23, Cold	October 15, Exercise	July 20, Control	September 23, Cold	
per cent	*per cent*	*per cent*	*per cent*	*per cent*	
0.144	0.118	0.114	0	0	Before feeding
0.270	0.228	0.111	4.50 (13 cc.)	Slight	1 hour after feeding
0.149	0.115	0.100	1.85 (84 cc.)	0	3 hours after feeding
0.112	0.100		0	0	6 hours after feeding

Dog B2–02 was known to have very mild latent diabetes, but was continuously free from glycosuria on bread diet. Tests similar to those of dog B2-01 were carried out with the giving of 30.5 grams Merck glucose (3 grams per kilo) by stomach tube. Cold seemed to reduce hyperglycemia and glycosuria as compared with the control day. On the exercise day there was by far the lowest plasma sugar and no glycosuria.

Dog D4–29

PLASMA SUGAR			TIME
October 4, Control	October 9, Cold	October 10, Control	
per cent	*per cent*	*per cent*	
0.093	0.120	0.147	Blood before feeding
0.307	0.357	0.256	1 hour after feeding
0.307	0.455	0.202	2 hours after feeding
0.322	0.400	0.235	3 hours after feeding
0.307	0.416	0.222	4 hours after feeding
0.313	0.364	0.250	5 hours after feeding
	0.322	0.206	8 hours after feeding
	0.125	0.200	13 hours after feeding

Dog D4–29. Female; mongrel; age 2 years; good condition; weight 11.5 kilos. September 28, 1916, removal of pancreatic tissue weighing 26.9 grams. Remnant about main duct estimated at 3.8 grams (⅛). The removal of 0.65 gram additional tissue on October 16 was necessary to produce diabetes. In the interval the tests shown in the table were performed, with the feeding of 200 grams bread and 150 grams glucose on each occasion (together with 200 grams talcum powder as a precaution against diarrhea). On October 9 the dog was transferred to the cold room immediately after feeding. The plasma sugar curve ran noticeably higher on this day than on the control days, but yet was lower at the end of 13 hours, as if more of the available carbohydrate had either been excreted or consumed by shivering. An accident prevented comparison of the glycosuria.

Dog B2–86. The history of this animal was given in paper 2 of the preceding series (3). The animal had been on the verge of diabetes with an unusually large pancreas remnant, and enormous quantities of bread and glucose had been necessary to keep up glycosuria, but by July 21 this was disappearing and the appetite was failing. Therefore the expedient was adopted of raising the animal's metabolism to the highest level possible, by exercising him on the treadmill to the limit of his strength daily, and keeping him in the ice room all the rest of the time, in the endeavor to bring on diabetes either by stimulation of appetite or by any direct influence upon the pancreas. July 22 was occupied in this manner. July 23, exercise was deferred until a feeding experiment with 400 grams bread and 500 grams glucose could be carried out in the ice room in comparison with the one at summer temperature on July 21.

TIME	PLASMA SUGAR	
	July 21, at summer temperature	July 23, at freezing temperature
	per cent	*per cent*
Before feeding	0.124	0.100
2 hours after	0.147	0.128
7 hours after	0.141	0.141

The lower sugar curve on July 23 may have been partly the result of the preceding day's exercise, but at least augured failure for the undertaking. The program of combined exercise and cold was continued daily, with addition of as much as 600 grams of glucose to the bread diet, up to the time of the second operation on August 7. The strong animal merely thrived on the program, and such diabetic tendency as had seemed to be present disappeared.

Dog B2–71. June 3, 1914, at a normal weight of 14.7 kilos, nine-tenths of the pancreas were removed. In July, 1915 the dog was still in a state of moderate diabetes, kept sugar-free on meat diet, at a weight of 12.5 kilos, and was used for exercise and other experiments. Some tests were then performed with feeding 50 grams of bread, following only the urine without blood analyses. The regular lung diet was given each evening after completion of the test.

TIME	URINE		REMARKS
	Volume	Glucose	
	cc.	*per cent*	
July 28			
2:30 p.m.		0	Fed 50 grams bread
5:00 p.m.	38	0.28	
July 29			
2:30 p.m.		0	Fed 50 grams bread and placed in ice room
5:00 p.m.	47	0	Returned to summer temperature
July 30			
10:00 a.m.		0	Fed 50 grams bread and placed in ice room
5:00 p.m.	214	Trace	Returned to summer temperature
July 31			
10:00 a.m.		0	Fed 50 grams bread
5:00 p.m.	275	0.4	

In this experiment there was distinctly greater glycosuria on July 28 and 31 in warm summer weather than on July 29 and 30 at freezing temperature.

Dog C3–00. Female; fox terrier mongrel; white and brown; age 5 years; good condition; weight 4.5 kilos. May 6, 1915, removal of pancreatic tissue weighing 11 grams. Remnant about main duct estimated at 1.25 grams ($\frac{1}{10}$). As sometimes happens in small dogs, glycosuria was absent on full meat diet even with this small pancreas remnant. On May 15 a change to bread and soup promptly brought heavy glycosuria, which ceased with a return to meat diet on May 21. A fixed diet of 750 grams lung was then given daily, but was not always eaten completely. After continuous absence of glycosuria, the dog was transferred to the ice room on May 29, and in the following 24 hours excreted 0.45 per cent sugar in 375 cc. urine. Glycosuria then continued absent on the same diet as before, until on June 7 the dog was removed from the ice room. At summer temperature a return to bread and soup diet produced immediate heavy glycosuria. Accordingly, in this experiment cold failed to maintain glycosuria on meat diet in a dog which was demonstrably diabetic as proved by glycosuria on bread diet.

In August the same dog was tested with the aid of plasma sugar analyses on a regular diet of 500 grams lung and 50 grams suet. There was no glycosuria.

TIME	PLASMA SUGAR	REMARKS
	per cent	
August 10		
10:45 a.m.	0.135	Immediately after this bleeding, transferred to ice room
12:00 noon	0.122	
4:45 p.m.	0.141	
August 15		
12:30 p.m.	0.182	
August 16		
12:45 p.m.	0.098	
4:45 p.m.	0.112	Immediately after this bleeding, transferred from ice room to summer temperature
August 18		
12:00 noon	0.167	Summer temperature
5:25 p.m.	0.143	

Dog B2–88

PLASMA SUGAR					TIME
November 15, Control	November 18, Control	November 20, Cold	November 23, Cold	November 26, Exercise	
per cent	*per cent*	*per cent*	*per cent*	*per cent*	
0.128	0.189	0.131	0.122	0.162	Blood before feeding
	0.250	0.222		0.263	½ hour after feeding
	0.257	0.233		0.189	2 hours after feeding
0.286			0.271		4 hours after feeding

Dog B2–88, with mild diabetes, received test meals of 200 grams bread and 100 grams beef lung. The plasma sugar curve seemed to vary chiefly according to the initial figure, without much influence of any of the special measures employed. Thus, both figures on November 15 are a trifle higher than on November 23 in the refrigerator. Also the differences between November 18, 20 and 26 seemed to be governed chiefly by the level on which the plasma sugar started. Cold at least did not elevate the plasma sugar.

Dog B2–58. Female; mongrel; yellow; age 3 years; good condition; weight 11.8 kilos. Fasting was begun May 4, 1914, and by May 21 the weight was reduced to 9.1 kilos. It was thus possible to remove $\frac{11}{12}$ of the pancreas on this date with only a moderate degree of diabetes resulting. After various tests, the tolerance was spared by a low protein diet, which could be gradually increased by October 20 to 1 kilo of beef lung daily, without glycosuria. The weight gradually rose by December 24 to 12.3 kilos, on which date the first glycosuria appeared. About the same time the dog began to leave part of the diet uneaten, and the weight thus fluctuated and in general fell. The partial checking of glycosuria, and its daily variations, are thus accounted for. Beginning January 6, 1915, the dog's cage was kept outdoors, in order to test the effect of cold upon the diabetes either directly, through stimulation of appetite or in any other way. The dog had time to become acclimated because the weather was fairly mild at first, but after January 20 it turned decidedly colder, and there were several days with considerable snow and ice. As the dog was short-haired, she was shivering practically continuously while outdoors, but passed through even the coldest weather without actual impairment of health. The rectal temperature remained normal. Contrary to expectation, the amount of food eaten was not appreciably different in the cold environment. After February 12 the glycosuria was checked by fasting. The experiment seems to indicate a slight increase of glycosuria by cold, but the influence certainly was not great.

Dog B2–58

DATE	WEIGHT	URINE	
		Volume	Glucose
	kgm.	*cc.*	*per cent*
December 24		320	1.0
25		585	0.5
26	12.8	470	Faint
27		385	0.2
28		950	Faint
29		355	0
30	12.7	384	0
January 1		360	0
2		500	0
3		632	0
4		650	Faint

Dog B2-58—Concluded

DATE		WEIGHT	URINE	
			Volume	Glucose
		kgm.	*cc.*	*per cent*
January	5		480	0
	6	12.7	330	0
	7	12.4	502	0
		Today placed	in cage outdoors	
	8		431	0
	9		307	0
	10		575	0
	11	11.1	713	0
	12		766	0
	13	11.2	646	0
	14		954	0
	15	10.9	698	0
	16		382	0
	17	Not	collected	
	18	11.1	1849	0
	19		988	0 6
	20	11.3	995	0
	21		965	0.7
	22	11.1	781	0.9
	23	11.2	609	0.5
	24		740	0.9
	25	10.9	700	Faint
	26	11.1	749	0
	27	11 0	900	V. Faint
	28	11 3	731	1.5
	29	11.5	630	1.0
	30	11.5	156	2.0
	31		820	1.1
February	1	11.3	1036	1.0
	2	11.3	1300	0.8
	3	11.0	950	2.0
	4	11.1	785	1.8
	5	11.4	1157	2.0
	6	11.8	605	2.5
		Today moved into	animal room	
	7		650	1.0
	8	11 5	973	0.9
	9	11.5	649	0.4
	10	11.7	733	0.7
	11	11.5	815	Faint
	12		600	Faint

Dog B2–89. This female mongrel, weighing 13 kilos, underwent partial pancreatectomy on April 12, 1915, leaving a remnant of $\frac{1}{11}$. Under regulated diets, the condition by the end of June was such that glycosuria was absent at a weight of 10.3 kilos on a diet of 1 kilo of lung, but substitution of 250 grams lung by 50 grams bread (making 750 grams lung and 50 grams bread) caused glycosuria of 0.75 per cent in 462 cc. urine on June 30 and 0.71 per cent in 542 cc. urine on July 1. On the latter date the diet of 1 kilo of lung without bread was resumed, and glycosuria immediately ceased (4). On July 2 the dog was transferred to the ice room, in order to test whether the influence of cold would amount to as much as the above difference between carbohydrate and protein. The dog was left in the ice room until July 26. Traces of glycosuria were present on most days during this time, but only twice reached titratable amounts (0.33 per cent on July 14, 0.25 per cent on July 21). A single day of exercise on July 22 abolished the glycosuria, which returned on July 24. It was present also on July 25 and 26, and then was continuously absent during a control period up to August 5 at summer temperature. As the weight fell to 9.7 kilos during the period in the refrigerator, the experiment seems to indicate a slight increase of diabetic tendency due to cold. The difference due to temperature, however, was evidently less than the difference between the preformed carbohydrate of 50 grams of bread and its approximate equivalent of potential carbohydrate in protein. The tolerance had fallen somewhat, for the giving of 50 grams of bread on August 15 resulted in a glycosuria of 1.4 per cent.

Glycosuria remained absent on the lung diet to September 17. On that day at 9:30 a.m. the plasma sugar was 0.143 per cent, the rectal temperature 101.4° F. The dog was then placed in the ice room fasting. At 4 p.m. the plasma sugar was 0.151 per cent, the rectal temperature 100.7°F. The usual lung diet was then fed and the dog left in the ice room. The next morning there was a glycosuria of 0.42 per cent in 567 cc. urine, and the plasma sugar at 9:30 a.m. was 0.164 per cent. The urine of the next 24 hours was 492 cc., with 1.9 per cent sugar. At 9:30 a.m. on September 19 the rectal temperature was 101.5°F. Glycosuria then ceased abruptly, as though the reserve of extra carbohydrate had been exhausted. At 9:30 a.m. on September 20 the plasma sugar was 0.146 per cent. The dog was then transferred to the warm animal room, and at 9:30 a.m. on September 21 the plasma sugar was 0.133 per cent, the rectal temperature 101.7°F.

During the following days the weather turned colder. The night of September 23–24 was particularly sharp, and the door of the animal room blew open, so that the room was cold and the dogs all shivering. This dog was one of five potentially diabetic animals (out of about twenty) which had been kept sugar-free on regulated diets and which showed sudden glycosuria on this night.

Subsequently, at a body weight of 15 kilos and correspondingly reduced tolerance, a comparison was made in this dog of the feeding of 1 kilo of meat in a warm room and in the ice room.

TIME	PLASMA SUGAR	
	November 26, (warm room)	December 3, (ice room)
	per cent	*per cent*
Before feeding	0.200	0.208
2 hours after feeding	0.218	0.278
5 hours after feeding	0.208	0.313
Glycosuria for the period	0	4.08 grams

The later observations showed a decided influence of cold for the production of hyperglycemia and glycosuria. It is possible that this effect became greater as the diabetes became more severe.

Dog B2–79

PLASMA SUGAR		URINE SUGAR		TIME
November 23, Warm	November 30, Cold	November 23, Warm	November 30, Cold	
per cent	*per cent*	*per cent*	*per cent*	
0.133	0.250	0	Faint	Blood before feeding
0.213	0.416	Faint	5.00	1 hour after feeding
0.159	0.525	Faint	5.90	4 hours after feeding
0.189	0.435	0	3.53	6 hours after feeding

Dog B2–79 was an animal which had long been kept in a stage of diabetes such that there was marked hyperglycemia and occasional glycosuria on a diet of 1 kilo of beef lung, the condition being held in check by fasting when necessary. The above record shows a strong contrast in hyperglycemia and glycosuria on days spent in a warm room and in the ice room respectively. The rise of blood sugar after eating the usual kilo of lung was somewhat similar on the two days, but the entire curve was on a much higher level in the cold environment. It was not established that this difference was due entirely to the temperature, because no comparison was made of the blood sugar before and after moving into the refrigerator on November 30. Therefore on December 2 the plasma sugar was determined fasting at 11:40 a.m. and found to be 0.164 per cent. The dog was then moved into the cold room, and at 5:40 p.m. the plasma sugar (still fasting) was found to be 0.185 per cent.

Likewise dog C3–19, weighing 13.8 kilos, was partially depancreatized on June 23, 1915, leaving a remnant of $\frac{1}{11}$ to $\frac{1}{12}$. In August at a weight of 11 kilos, the degree of diabetes was such that a diet of 500 grams lung was slightly in excess of the tolerance. On August 7 the fasting plasma sugar at 10:30 a.m. was 0.156 per cent and at 4:30 p.m. 0.120 per cent. The dog, still fasting, was then moved to the ice room, where the plasma sugar was found to be 0.167 per cent at 7 p.m. and 0.133 per cent at 9:45 p.m. Here a falling blood sugar due to fasting was evidently raised by cold, and then continued to fall slightly.

DISCUSSION

The elevation of blood sugar by cold is so generally accepted as a truism that it was a surprise to encounter instances in which the sugar was little changed or actually lower in a cold environment. It was not feasible to extend the investigation further into the physiological reaction to cold. Supposedly the blood sugar is governed by two factors, namely the mobilization and the disposal of carbohydrate. It is conceivable that in a perfectly smoothly working reaction the two may exactly balance. Under some conditions shivering may perhaps reduce the blood sugar like other forms of muscular activity. Under other conditions hyperglycemia may occur, particularly when the cold stimulus is sufficiently violent, as for example in the case of plunging into ice water. Here the stimulation is so powerful, sometimes to a pathological degree, that a correspondingly excessive sugar discharge may be expected, and in the most extreme cases possibly the utilization of sugar suffers somewhat. Hyperglycemia and glycosuria may be more readily produced when the utilization of sugar is specifically impaired, as in the more severe grades of diabetes. In a similar way exercise sometimes raises the blood sugar instead of lowering it.

Violent or pathological stimulation by cold was not applicable in experiments designed to produce diabetes, because the animals' health would suffer and ill health would be the surest way to spoil the result and prevent diabetes. A more powerful temporary discharge of sugar might occur, but it would cease as soon as the immediate store was exhausted. Such discharge and cessation was actually seen in certain of these experiments, without any lasting diabetogenic effect. As usual, clear thinking requires a distinction between diabetes, which is deficiency of the pancreatic function, and mere glycosuria. The mere excessive discharge of sugar from the glycogen depots is not diabetes, for the power of utilization may remain unimpaired. This has been abundantly proved, for example, in such a condition as epinephrin glycosuria (5). It is also not diabetes if the utilization of sugar is depressed by any extraneous mechanism, such as the chilling of the muscles or their nervous supply, but only if the impairment of utilization is due to impairment of the pancreatic function. If the glycosuria produced by cold is regarded as a diabetes, cold must be a very powerful diabetogenic agent to cause even a temporary diabetes in a normal animal, for it must thus temporarily paralyze about nine-tenths of the function of the dog's pancreas. Trial of this agent in animals depancreatized almost to the point of diabetes proves that it possesses no such power; and as the effect in these animals is not greatly different from that in normal animals, cold evidently does not act by direct depression of the pancreatic function. Its indirect influence upon diabetes through increasing metabolism is a much more delicate point to demonstrate, and the question is answered only somewhat doubtfully in the affirmative by some of the above experiments.

CONCLUSIONS

1. Cold environment, such as did not lower the rectal temperature to any important extent, in some instances failed to affect the plasma sugar of dogs or slightly lowered it, but in the majority of experiments produced hyperglycemia and sometimes glycosuria. These were produced more easily and in higher degree in proportion as the power of sugar utilization was impaired, i.e., as the diabetes was more severe.

2. The power to produce glycosuria is to be distinguished from the power to produce diabetes. There is no demonstrable difference in the proportion of pancreatic tissue that must be removed to produce diabetes in dogs in warm or cold environment, and it was proved by successive operations upon the same animals that the influence of cold is not equivalent to the removal of the smallest fraction of a gram of pancreatic tissue. In animals already diabetic, the course of the diabetes in a few instances seemed to be influenced slightly for the worse, so as perhaps to warrant the conclusion that cold imposes an increased burden upon the pancreatic function by increasing metabolism. But the slightness of this influence is emphasized by control experiments; for example, it amounts to less than the difference between the preformed carbohydrate of 50 grams of bread and the approximate equivalent of potential carbohydrate in protein.

3. The impression that diabetic patients do worse in cold weather is probably explainable by the discomfort of chilliness when they are undernourished, the tendency to take more food, and sometimes by the limitation of exercise. These may be important sometimes from a practical standpoint, but any direct influence of climate upon diabetes must be very slight if it exists.

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EXPERIMENTAL STUDIES IN DIABETES

SERIES II. THE INTERNAL PANCREATIC FUNCTION IN RELATION TO BODY MASS AND METABOLISM

8. The Influence of Extremes of Age upon the Production of Diabetes

FREDERICK M. ALLEN

From the Hospital of The Rockefeller Institute for Medical Research, New York

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An influence upon the production of diabetes may conceivably be expected from the conditions of extreme age or youth.

Senility. The relation of senility to experimental diabetes may have interest from at least three standpoints: *a*, The total metabolism is known to be slightly lowered in old age; *b*, diabetes in elderly patients is generally characterized by a mild and prolonged course; *c*, the increasing incidence of diabetes with the advance of age suggests the possibility of functional or organic impairment of the pancreas.

Toothless decrepit senility is familiar in dogs, and there is reason to expect as great metabolic changes as in aged human beings. Obesity is also common in such animals, so that a number of them had to be included in a previous paper (1). A comparison of the relation of pancreas weight to body weight was made in fourteen dogs, which showed extreme senility together with an average nutritive state.

From a comparison of table 1 with a similar study of normal adult dogs (2), it may be inferred that there is no gross change, in particular no atrophy of the pancreas accompanying senility.

The susceptibility of obese senile dogs to diabetes could seldom be determined, owing to the sudden death to which they are subject following pancreas operations, as previously mentioned (3). Such operations were performed without accidents in senile dogs without obesity, and these were used for diabetic experiments in the same way as younger dogs. No experiments were performed to determine the precise proportion of pancreas that must be removed to produce diabetes, but the incidental observations were sufficient to exclude any marked differences from average adult dogs. The senile animals were somewhat weaker and more subject to loss of appetite and cachexia. Otherwise their diabetes ran a course indistinguishable from that of younger animals. The experimental answers may therefore be stated in the following form, corresponding to the questions raised above.

TABLE 1

Relation of pancreas weight to body weight in senile dogs

NUMBER	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS PER KILOGRAM BODY WEIGHT
	kgm.	*grams*	*grams*
1	4.0	15.0	3.75
2	4.4	7.0	1.59
3	5.8	9.5	1.64
4	8.8	23.1	2.62
5	11.3	19.6	1.73
6	11.3	24.4	2.16
7	11.5	25.6	2.23
8	11.7	32.1	2.74
9	14.2	53.6	3.78
10	15.0	33.6	2.24
11	16.0	20.2	1.26
12	21.3	46.4	2.18
13	21.8	39.6	1.81
14	25.1	41.4	1.65

a. The lowered metabolism characteristic of senility does not render dogs less susceptible to diabetes from pancreatic resection. As the reduction of metabolism with age is slight, and the method of judging susceptibility by the size of the pancreas remnant with which diabetes occurs is a crude one according to former observations, too much stress need not be laid upon these negative findings.

b. A more decisive observation is that diabetes does not run any slower course in senile animals, but follows the same rapid progress which is generally characteristic of diabetes in dogs.

c. If there were any anatomic or functional deterioration of the pancreas with age, a remnant of a given size might be expected to be less efficient in preventing diabetes than in younger animals. An increased susceptibility to diabetes in this sense is excluded by the observations. The microscopic study, as reported in subsequent papers, showed no visible abnormalities resulting from simple senility. Pancreatitis is much rarer in dogs than in human beings, and is apparently due to causes independent of age. Also there is no such arteriosclerosis in senile dogs as in man, and it can by no means be said that a dog "is as old as his arteries." Also, as previously mentioned (2), the pancreas remnant of a senile dog may possess as great power of hypertrophy as that of any younger animal.

Numerous glucose tolerance tests have been performed indiscriminately upon old and young adult dogs, and no indications of alteration of assimilation with age have been found.

In general these observations, made upon a species practically free from the pancreatic changes to which man is subject with advancing years, indicate that the rising incidence and special characteristics of diabetes in older persons are due to the changes in question and not to senility *per se*.

Youth. The outstanding feature of experimental interest is the characteristically rapid and fatal course of diabetes in children. This has often been attributed hypothetically to their high metabolism, which imposes a heavier burden upon the pancreatic function. There is also good evidence that this rapid downward progress indicates a susceptibility of the islands of Langerhans to rapid destruction by hydropic degeneration. As there are occasional cases of acute and severe diabetes in the aged and of mild and prolonged diabetes in children, there is no absolute distinction on the basis of either the level of metabolism or the island changes.

Diabetes is also less frequent in children than in older persons. In seeking possible reasons, it might be imagined that the youthful pancreas is larger in proportion to the body, that it is anatomically richer in islands or that these have stronger functional power, or that the capacity for regeneration after injuries is greater. According to work previously reviewed (4), especially that of Bensley, the pancreas at birth probably contains as many islands as the adult organ, but during early life there is probably a loss followed by a gradual new formation of islands. Observations on the gross relations of the pancreas in puppies are contained in tables 2 to 6. They indicate, in comparison with those on adult dogs (2), that the ratio of pancreas weight to body weight in puppies is not large but rather small in proportion to what might be expected from the small size of the animals; that the tendency to regeneration is often marked but yet not in excess of that often found in adults; and that the tendency to diabetes is at least no greater and often is distinctly less than in adult dogs.

In qualification of this statement, it should be noticed that the observations do not exclude possible alterations of the ratio of pancreas

TABLE 2

Litter of black and tan mongrel pups. Weight of mother, 17 kilos

NUMBER	SEX	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM
			kgm.	*gram*	*grams*
1	Male	Newborn	0.3	0.6	2 0
2	Male	Newborn	0.3	0.6	2 0
3	Female	Newborn	0.2	0.4	2.0
4	Female	Newborn	0.3	0.5	1.67
5	Male	Newborn	0.3	0.6	2.0
6	Female	Newborn	0.3	0.7	2.33
7	Male	Newborn	0.4	0.8	2.0
8	Female	Newborn	0.3	0.9	3.0
9	Male	Newborn	0.3	0.6	2.0
10	Male	Newborn	0.3	0.8	2.66
11	Female	Newborn	0.3	0.6	2.0

TABLE 3

Litter of harrier mongrel pups. Weight of mother 13 kilos

NUMBER	SEX	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM
			kgm.	*gram*	*grams*
1	Female	1 day	0.2	0.3	1.5
2	Male	1 day	0.2	0.3	1.5
3	Male	1 day	0.3	0.6	2.0
4	Male	1 day	0.3	0.4	1.33
5	Male	1 day	0.3	0.7	2.33
6	Male	1 day	0.2	0.7	3.50

TABLE 4

Litter of spaniel mongrel pups. Weight of mother, 16 kilos

NUMBER	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM
		kgm.	*grams*	*grams*
1	Slightly premature	0.2	0.7	3.5
2	Slightly premature	0.2	0.6	3.0
3	Slightly premature	0.3	0.8	2.67
4	Slightly premature	0.3	0.6	2.0
5	2 days	0.3	0.9	3.0
6	3 weeks	0.6	2.1	3.5

weight to body weight with age. In other words, there is no proof that a puppy having a certain ratio will maintain this same ratio up to adult age. In tables 4 and 5 no great change was noticed in the ratio up to 2 or 3 weeks of age, using other pups of the same litter as controls. The ratios varied widely among the different pups in table 6. They were sometimes larger in the smaller breeds, as found for adult dogs (2), but the rule was not uniform. The state of nutrition

TABLE 5

Litter of yellow and brown mongrel pups. Weight of mother, 21.5 kilos

NUMBER	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM
		kgm.	*grams*	*grams.*
1	½ week	0.3	1.1	3.67
2	½ week	0.4	1.7	4.25
3	½ week	0.4	1.3	3.25
4	2 weeks	0.6	2.6	4.33
5	2 weeks	0.7	2.8	4.00

TABLE 6

Relation of pancreas weight to body weight in puppies

NUMBER	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM
		kgm.	*grams*	*grams*
1	1 week premature	0.1	0.2	2.0
2	1 week premature	0.1	0.2	2.0
3	1 month	0.7	2.2	3.14
4	1 month	1.5	7.1	4.74
5	1½ months	2.1	4.1	1.95
6	1½ months	1.3	2.6	2.00
7	2 months	0.9	3.5	3.89
8	2 months	2.3	5.1	2.22
9	2 months	2.0	5.9	2.95
10	2 months	1.8	5.8	3.22
11	2 months	4.3	8.4	1.95
12	2 months	2.3	7.4	3.21
13	2½ months	1.8	7.4	4.10
14	2½ months	2.5	5.7	2.28
15	3 months	2.5	12.2	4.89
16	3 months	2.3	5.2	2.26
17	5 months	3.9	13.8	3.54
18	7 months	2.5	11.4	4.55

TABLE 7

Partial pancreatectomies in puppies

NUMBER	AGE	BODY WEIGHT	PANCREAS WEIGHT	PANCREAS WEIGHT PER KILOGRAM	WEIGHT OF REMNANT	SIZE OF FRACTION	HYPERTROPHY	REMARKS
	months	*kgm.*	*grams*	*grams*	*grams*		*grams*	
1	1	3.0	13.2	4.4	0.8	1/16–1/17	0.8–1.02	
2	2	1.9	9.7	5.1	1.3	1/7–1/8	1.3–5.7	Transitory glycosuria. Mild diabetes produced by removal of additional 1.2 grams
3	2	1.7	8.9	5.2	0.7	1/13–1/14	0.7–0.4	Cachexia. Weight 1 kgm. at autopsy. Probably mild diabetes
4	3	2.5	11.0	4.4	0.5	1/22	0.5–0.6	Cachexia. Mild diabetes
5	3	2.1	10.8	5.1	3.0	1/3–1/4		Diabetes not produced even by circulatory stasis
6	3	3.9	17.8	4.6	5.7	1/3		Diabetes not produced even by circulatory stasis
7	3	2.0	16.0	8.0	2.7	1/6	2.7–5.3	No diabetes
8	3	2.1	8.3	3.9	0.8	1/10		Thin, no glycosuria
9	4	3.7	15.8	4.3	1.5	1/10		Assimilated 5 grams of glucose per kilogram
10	4	8.4	27.6	3.3	4.6	1/5		Removal of 3.6 grams additional tissue in 6 operations required to bring on diabetes. Remnant at autopsy was then 1.1 grams
11	5	1.9	7.8	4.1	0.6	1/3	0.6–1.0	
12	5½	5.3	25.8	4.9	4.4	1/6	5.4–8.4	No diabetes
13	6	8.0	22.5	2.8	9.3	1/2–1/3		
14	6	3.8	8.5	2.2	1.0	1/8–1/9		
15	7	5.9	20.1	3.4	1.7	1/12		Peritonitis, no glycosuria
16	7	5.9	15.0	2.54	1.6	1/9–1/10		Diabetes stopped by distemper
17	7	3.4	9.3	2.74	1.5	1/6		Cachexia, no glycosuria
18	7	4.4	8.9	2.02	1.0	1/10		Diabetes, mild, transitory
19	8	7.2	19.8	2.75	1.8	1/11		

TABLE 7—*Concluded*

NUMBER	AGE	BODY WEIGHT	PANCREAS, WEIGHT	PANCREAS WEIGHT PER KILOGRAM	WEIGHT OF REMNANT	SIZE OF FRACTION	HYPERTROPHY	REMARKS
	months	*kgm.*	*grams*	*grams*	*grams*		*grams*	
20	9	5.8	21.5	3.71	1.3	$\frac{1}{17}$		Diabetes prevented by preliminary fast
21	9	2.7	10.5	3.90	1.0	$\frac{1}{10}$		Peritonitis, no diabetes
22	9	7.4	24.8	3.36	2.5	$\frac{1}{10}$		Severe diabetes, even during fasting
23	9	7.1	26.2	3.69	3.0	$\frac{1}{9}$	3.0–1.95	Severe diabetes checked by cachexia. Emaciated to 5.3 kgm.
24	9	10.3	30.9	3.00	3.1	$\frac{1}{10}$	Considerable	
25	11	4.1	14.3	3.50	1.1	$\frac{1}{18}$	1.1–2.2	Cachexia, no diabetes

or rate of growth is probably an important factor. The differences found among animals of the same litter at the same age in tables 2 and 3 interfere seriously with studies based on any method of controls.

Partially depancreatized puppies are especially liable to cachexia from respiratory infections or diarrhea, so that diabetes is often thus suppressed and the experiments spoiled. The above statement concerning the relatively slight disposition to diabetes is based on animals which remained vigorous and thriving after operation. It is the more striking in view of the fact that the sugar tolerance of the young is distinctly less than that of adult normal animals (5). A series of observations was made upon the question whether partial pancreatectomy short of diabetes interferes with the growth or health of puppies, with negative results except for the temporary backset due to the operation, and a possible specific inhibition of development of sexual and other adult characters. Anything in the nature of a masked diabetes or other fatal metabolic deficiency seemed to be excluded.

As explained in a subsequent publication on acidosis, the expectation that puppies might prove more susceptible than adult dogs to diabetic acidosis was entirely disappointed. Acidosis was absent throughout the reported experiments except where specially mentioned.

As puppies approach adult age, they approach more closely to the adult behavior respecting diabetes. After about the ninth month they generally react like adults except for susceptibility to distemper. Nevertheless in pup 18 in table 7 it is noticeable that diabetes was transitory with a pancreas remnant of only $\frac{1}{10}$. Some typical records of younger puppies are the following.[1]

No. G7-49. Male; mongrel; black and white; age 1 month; well nourished; weight 3 kilos. June 25, 1918, removal of pancreatic tissue weighing 12.4 grams. Remnant about main duct estimated at 0.8 gram ($\frac{1}{16}$ to $\frac{1}{17}$). Following operation there was diarrhea and loss of weight but no glycosuria, though the pup lived on bread and milk. July 4, 0.32 gram additional pancreatic tissue was removed. The condition was still cachexia without glycosuria, up to death on July 11. The pancreas remnant weighed 0.7 gram, and was too badly autolyzed for microscopic study. In the tissue removed on July 4, the acini were normal and well filled; islands were present in normal number and size but showed slight distinct vacuolation. This hydropic change makes it probable that hyperglycemia was present, and possibly only the animal's weakness prevented a frank diabetes. Acidosis was absent as usual.

No. D4-35. Female; mongrel; age 3 months; brown and white; well nourished; weight 2.5 kilos. November 9, 1916, removal of pancreatic tissue weighing 10.5 grams. Remnant about main duct estimated at 0.5 gram ($\frac{1}{22}$). Glycosuria was brought on by the feeding of 20 grams meat and 20 cc. milk, but ceased readily with fasting. Death occurred from weakness, notwithstanding a low meat diet, on November 20. The pancreas remnant weighed 0.6 gram, and was not examined microscopically. The pup obviously had a potentially severe diabetes, but differed from an adult dog in that glycosuria did not begin spontaneously and was promptly checked by fasting and low diet, with such a very small pancreas remnant.

No. D4-34 similarly underwent removal of 8.2 grams pancreatic tissue, leaving a remnant estimated at 0.65 gram ($\frac{1}{13}$ to $\frac{1}{14}$). Glycosuria could readily be checked by fasting, but on full meat diet was as high as 1.2 per cent. Notwithstanding the liveliness following operation and the meat diet, the weight and strength failed rapidly. The animal was killed when moribund on the tenth day after operation, when the plasma sugar was 0.05 per cent. The rapid cachexia was the chief point of difference from an adult dog.

No. G7-25. Female; Boston terrier; brindle; age 6 weeks; good nutrition; weight 3.8 kilos. June 17, 1918, removal of pancreatic tissue weighing 13.1 grams. Remnant about main duct estimated at 1.1 gram ($\frac{1}{13}$). Glycosuria remained absent, first on beef lung, then on bread and soup diet, then with addition of 50 grams glucose. June 25, at a weight of 3.5 kilos, 1 gram additional pancreatic tissue was removed, the remnant having undergone marked hypertrophy. Glycosuria remained absent on bread and milk diet, though weight was lost. July 4, 0.95 gram additional pancreatic tissue was removed. The animal was lively and ate considerable lung on July 5, then gradually lost appetite and weight while retaining spirits up to death on July 9. Glycosuria remained absent. The plasma sugar on June 17 was 0.143 per cent, on July 8, 0.162 per cent. The pancreas remnant weighed 0.8 gram. In the various specimens of pancreas from June 17 and July 4 and 9, inflammation and fibrosis were limited to the peripheral areas. In the central portions the acini were well filled, sometimes large and irregular. Islands were normal in number and free from vacuolation. Here the persistent absence of diabetes is perhaps attributable to the hypertrophy of the pancreas remnant.

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[1] All operations were performed under ether anesthesia.

No. E5–40. Male; mongrel, part Newfoundland; age 3½ months; good nutrition; weight 8.4 kilos. May 14, 1917, removal of pancreatic tissue weighing 23 grams. Remnant about main duct estimated at 4.6 grams (⅕). Glycosuria was absent on bread and soup diet, and only transitory with addition of 100 grams glucose.

May 24, additional pancreatic tissue weighing 0.9 gram was removed. There was obvious hypertrophy of the remnant in all three dimensions. Thereafter glycosuria was absent on bread diet, and could be maintained only by increasing additions of glucose, first 50, then 100, then 200 grams daily. The dosage of 200 grams was continued from June 6 to 27 with only traces of glycosuria and with a gain of weight to 9.3 kilos.

June 27, 0.6 gram additional tissue was removed from the pancreas remnant. which again was obviously hypertrophic. Thereafter heavy but transitory glycosuria resulted first from 100 and then from 200 grams glucose added to the bread diet.

July 12, an additional 0.35 gram of pancreatic tissue was removed, the weight then being 9.8 kilos. Glycosuria was then heavy with addition of 200 grams glucose to the bread diet, but ceased July 19.

July 20, an additional 0.4 gram of pancreatic tissue was removed. Glycosuria was then heavy on bread and soup diet, next with addition of glucose up to 200 grams, but was absent after July 29.

August 31, at a weight of 10.6 kilos, glycosuria being still absent on the bread diet with 200 grams glucose, an additional 0.52 gram of pancreatic tissue was removed. Glycosuria was again transitory.

September 7, an additional 0.3 gram of pancreatic tissue was removed with a similar result, glycosuria ceasing September 18.

September 28, an additional 0.55 gram of pancreatic tissue was removed. Glycosuria was then continuous, first with glucose, then on plain bread and soup, till stopped by a change to meat diet on October 22. It may be noted that the downward progress was slow rather than rapid, for the glycosuria of many adult dogs after 3 weeks reaches a point where it cannot be stopped by fasting.

The pup did not thrive on carbohydrate-free diet, suffered from indigestion, diarrhea and loss of weight, and died in cachexia November 16 at a weight of 7 kilos. The blood sugar was low during this period, and there was the usual absence of acidosis.

The pancreas remnant weighed 1.1 gram. Nothing significant was found in the gross autopsy, or in the microscopic examination of the liver, kidneys, adrenals, thyroid and parathyroids. Sections from the pancreas at all the operations and at autopsy showed normal parenchyma and absence of hydropic degeneration. Though the hypertrophy may have gone far toward preventing diabetes, it is evident from the repeated operations and the prolonged carbohydrate excess that this puppy was difficult rather than easy to make diabetic.

No. D4–21. Male; mongrel; age 7 months; good nutrition; weight 6.7 kilos. September 15, 1916, removal of pancreatic tissue weighing 24 grams. Remnant about main duct estimated at 2.9 grams (⅛). Glycosuria at first was absent on moderate quantities of milk, but was heavy on bread feeding September 18. It was then checked by fasting, and kept absent on a diet of lung, suet and 100 grams bread. The animal thrived and máintained a weight of about 7 kilos.

December 5, a full bread diet was resumed, and heavy glycosuria returned promptly. After 4 days this was stopped as before. The animal grew to adult life on a diet of 400 grams lung, 100 grams suet, and part of the time 100 grams bread or 100 cc. milk. Yeast was added part of the time, with the idea that it and the milk might supply needed vitamines; also an admixture of bonemeal assured adequate salts. The animal became plump and strong at a weight of 8.7 kilos, but remained always undeveloped in mentality, in the size of the sexual organs and the absence of any apparent sexual function; urination was performed squatting, and the puppy contour of the body and puppy-like behavior toward other dogs were retained. Pratt (6) has made similar observations in young dogs with pancreatic atrophy.

November 16, 1917, it was found that the addition of 100 grams bread to the diet produced a glycosuria of 1.3 per cent in 820 cc. of urine. The tolerance was thus evidently lower than before, and thereafter the diet was kept carbohydrate-free (400 to 600 grams of lung and 100 grams of suet). April 6, 1918, the fasting plasma sugar was found to be 0.250 per cent, indicating that hyperglycemia was now continuous. The weight at this time was 9.15 kilos. Traces of glycosuria began in July, at first intermittently, but by July 30 they were continuous and increasing, so that fasting had to be used. The weight at this time had reached its maximum of 9.5 kilos. The animal appeared not obese but in excellent nutrition.

The diet was then gradually built up to 200 grams lung and suet ad libitum, without glycosuria. This diet continued while the writer was in military service. At a visit on September 16, 1918, the animal was found in excellent strength and spirits, weighing 8.3 kilos, with intense glycosuria and hyperglycemia and heavy nitroprusside reactions in urine and plasma. Notwithstanding the fat-rich diet and acidosis, there was no visible lipemia. The condition was ideal for the development of coma, but under the circumstances there was nothing to do but to kill the animal for autopsy.

The body appeared in excellent condition and retained much fat. Except for a very fatty liver and the puppy-like characters noted above, the gross autopsy was negative. The pancreas remnant weighed 3.1 grams. The practical absence of hypertrophy may be noticed.

Microscopically, the liver was crammed with fat even to the periphery of the lobules. Stains with Best's carmine showed absolutely no glycogen except in occasional leukocytes, which stood out prominently in the capillaries by reason of their stuffing of red granules.

The kidneys were normal except for maximal Armanni vacuolation, as seen in routine Zenker specimens stained with methylene blue and eosin. Best's carmine applied to the alcohol fixed specimens showed only a sparse sprinkling of glycogen, so that the vacuoles evidently represented chiefly fat deposit, as demonstrated by fat stains in some other animals but not in this one.

The adrenals showed no more than the average lipoid vacuolation in the cortex, and were normal in other respects.

The thyroid and parathyroids were normal, with average colloid content in the former.

The pancreas remnant was normal in structure and fullness of acini and number and size of islands, but a minority of island cells were maximally swollen and vacuolated.

This puppy, in respect to "spontaneous" downward progress and other features, behaved practically like an adult animal, and most of the history actually represented an adult period of life. Many long experiments of this character were begun for the purpose of testing whether a partial pancreatectomy which did not suffice to produce immediate diabetes in a puppy might result in diabetes after a certain stage of growth had been reached. In other words, the problem was whether a damaged pancreas might lag behind in development so as finally to become inadequate for the demands of a growing body. The best observations would have been those upon much younger animals, but all these failed for one cause or another. It is evident that in many cases the hypertrophy of the pancreas remnant fully compensates for any bodily growth. It is also very doubtful whether an animal which is actually non-diabetic will become diabetic by simple growth. But when the injury results in such a mild diabetes that there are no symptoms on whatever dietary régime is followed, it is entirely possible that diabetes may develop openly at a much later period under the strain of growth and gain of weight. Such a result is exemplified in this experiment, which covers a rather long period in proportion to a dog's life. Such experimental evidence makes it easily comprehensible that diabetic symptoms may follow either soon or late after an injury of the human pancreas; in particular, that glycosuria may be present immediately after an infection, or may be delayed to a time when there is no plain clinical connection with a long antecedent infection which may have been the real cause.

CONCLUSIONS

1. An influence of senility upon carbohydrate assimilation or diabetes is conceivable from a quantitative reduction or other alterations of metabolism, or from functional or anatomic changes in the pancreas. The observations upon senile dogs failed to show any departures from the normal in the glucose tolerance, the ratio of pancreas weight to body weight, the microscopic structure of the pancreas, the size of the pancreatic remnant with which diabetes occurs, the capacity of such a remnant for hypertrophy, or the clinical course of the diabetes.

2. An investigation was also made of the possible influence of the elevated metabolism or the pancreatic peculiarities of youth. Previous work had indicated that the glucose tolerance of puppies is less than that of adult dogs. No exact studies were made of the most important point in the microscopic anatomy, namely the richness in islands, but no striking departure from the adult average was noticeable in numerous routine observations. The ratio of pancreas weight to body weight was somewhat irregular, and the question of possible changes in an individual during growth could not be accurately decided, but the general average in puppies did not differ appreciably from that in adults, particularly with consideration of the small body weight. The remnant left after partial pancreatectomy generally grows considerably in puppies; the hypertrophy on the whole is greater than in adult dogs, but does not surpass what is found in occasional mature or adult animals, and may be slight or absent especially in older puppies. The tendency to diabetes is distinctly less in puppies than in adult dogs, partly on account of weakness and cachexia, partly because of hypertrophy of the pancreas remnant, and perhaps sometimes because of a high functional efficiency of a small remnant. There is no specific tendency to rapidity of downward progress or to diabetic acidosis in puppies.

3. An example is given of long delayed onset of diabetic symptoms on a fixed diet after gain of weight, this gain being largely growth instead of mere obesity. With a still milder diabetic tendency it is readily conceivable that the delay might be longer and might occur on an ordinary diet, also that the diet might in large measure determine the onset of symptoms. In this way it is possible that childhood infections injuring the pancreas may be responsible for some cases of diabetes which make their appearance at a much later period.

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EXPERIMENTAL STUDIES IN DIABETES

SERIES II. THE INTERNAL PANCREATIC FUNCTION IN RELATION TO BODY MASS AND METABOLISM

9. The Influence of Pregnancy upon Experimental Diabetes

FREDERICK M. ALLEN

From the Hospital of The Rockefeller Institute for Medical Research, New York

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Diabetes may conceivably be aggravated by the increased metabolism of pregnancy, which involves both additional food assimilation and the formation of considerable new tissue. Unknown toxic or metabolic factors may possibly have an influence. An opposite possibility is suggested especially by the work of Carlson and collaborators (1), namely, that diabetes in the latter part of pregnancy may be prevented by the internal secretory activity of the fetal pancreas. The chief question here is whether internal secretions pass in appreciable quantities through the placenta. In clinical practice pregnancy has long been regarded as decidedly injurious, to such an extent that therapeutic abortion was commonly recommended in women suffering from any serious grade of diabetes. The most comprehensive and recent survey of clinical experience is by Joslin (2), who considers that the supposed injurious effects are largely explained by the higher diet taken. One case of apparent gain of tolerance during pregnancy (3) is inconclusive, partly because the blood sugar rose during gestation and partly because it is uncertain whether the changes observed were due strictly to the pregnancy.

Partially depancreatized animals offer the most accurate obtainable conditions for studying the effect of pregnancy upon the internal pancreatic function. Before proceeding to the principal experiments, it is desirable to mention briefly some controls and unsuccessful attempts relating to this problem.

One control to be thought of is the possible effect of changes in the sexual organs themselves upon the pancreas or its function. This is part of the larger question of the interrelation of the sex glands with the pancreas and diabetes. A few extirpation experiments were performed as follows.[1]

Dog B2–89, a female mongrel aged 4 years, had been close to the verge of diabetes since the first operation on April 12, 1915, so that the removal of 0.25 gram additional pancreatic tissue on March 16, 1916 brought on mild diabetes. By repeated tests up to May 18 it was proved that glycosuria was regularly absent on a measured bread and soup diet, and was present to the amount of about 5 grams daily with the addition of 75 grams glucose. May 18, both ovaries were removed, together with the tubes and part of the uterine horns. A trace of glycosuria followed the operation, and another trace when the regular bread diet was fed. The addition of 75 grams glucose resulted in glycosuria of 12.5 grams one day and 13 grams another day. By May 27 glycosuria was again absent on the plain bread and soup diet and the glycosuria from glucose was no higher than before operation. The absence of any perceptible influence upon the diabetes, other than the transient effects of the trauma, was also confirmed by the animal's later history.

Dog D4–62. Female; mongrel; long haired; age 6 to 8 years; well nourished; weight 19.2 kilos. December 16, 1916, removal of pancreatic tissue weighing 27.5 grams. Remnant about main duct estimated at 3.2 grams ($\frac{1}{10}$). After fasting, glycosuria was found consistently absent on a diet of 1 kilo of beef lung, and moderate (0.5 to 1 per cent) with addition of 50 grams bread. January 17, 1917, both ovaries were removed. A trace of glycosuria followed the operation, but ceased on the lung diet. With addition of 50 grams bread it was distinctly greater than before (2.8 to 4.8 per cent, in larger urine volumes), but ceased when the bread was omitted on January 26. Thereafter the diabetic tendency was no greater than before the oöphorectomy.

Dog C3–67. Male; Dalmatian; age 1½ years; in excellent nutrition; weight 17.6 kilos. March 14, 1916, castration, and removal of pancreatic tissue weighing 28.5 grams. Remnant about main duct estimated at 2.6 grams ($\frac{1}{14}$ to $\frac{1}{15}$). The dog was used for a few tests (especially feeding of pure fat or talcum powder, as described elsewhere) which had no influence upon the glycosuria. Otherwise no food was given, but heavy glycosuria continued until death occurred from weakness on April 1, at a weight of 11 kilos. The pancreas remnant, weighing 3.1 grams, was normal except for the usual vacuolation of island cells. The bladder urine contained 0.61 per cent of sugar, and the autopsy otherwise was negative. Such irrepressible glycosuria with a pancreas remnant of this size is so unusual that the possibility of an aggravating influence of the castration was suggested.

Dog D4–63. Male; mongrel; long haired; age 3 years; medium nutrition; weight 15.25 kilos. After removal of pancreatic tissue on February 14 and March 7, 1917, glycosuria was maintained almost continuously on bread and soup diet with addition of 200 grams glucose to March 23, after which it was absent. Such a duration of glycosuria is evidence that an animal is very close to diabetes, which will be produced by the removal of a trifle more pancreatic tissue. The actual absence of diabetes was confirmed by blood sugar tests, the plasma sugar being

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[1] All operations were performed under ether anesthesia.

0.087 per cent before feeding and 0.145 per cent at the highest point afterward, and down to 0.122 per cent by evening even on the bread and glucose diet. March 28, castration was performed. Glycosuria remained absent on bread diet to April 2, and therafter also with addition of 200 grams glucose. The attempts to induce diabetes by diet were continued through May, with merely the usual slight gain in tolerance. The removal of 0.5 gram pancreatic tissue on May 8 proved inadequate, but mild diabetes followed the removal of 0.65 gram additional on June 1.

This single experiment with dog D4-63 probably suffices to prove that removal of the testes has no influence for or against the production of diabetes. Aggravation of an existing diabetes, which is a separate question, was not produced by removal of the ovaries in dogs B2-89 and D4-62. The apparent aggravation after removal of the testes in dog C3-67 may have been purely accidental, and any specific influence remains improbable. Circumstances prevented further experiments of this sort.

Numerous attempts were made to determine the size of pancreas remnant with which diabetes occurs in pregnant animals, with uniform failure on account of abortion or death. Efforts to immunize by one or several preliminary subcutaneous or intraperitoneal injections of aqueous extract of fresh sterile dog pancreas gave no protection. Trials were made with removal of five-sixths to nine-tenths of the pancreas in single operations, performed as quickly and easily as possible so as to be over in half an hour or less, but the animals always became unwell and aborted within a few days or at most a week. Such an interval, especially when glycosuria is prevented by malaise and refusal of food, can decide nothing with the pancreas remnants mentioned, though permitting some observations after total pancreatectomy as in Carlson's experiments. Other attempts were made with successive operations, in the hope that each might be so easy as to avoid abortion, also by leaving a duodenal remnant and a subcutaneous graft early in pregnancy, with the idea that diabetes might be brought on later in pregnancy by simple removal of the graft. All such attempts failed for one cause or another. It is sometimes possible to remove considerable portions of pancreas, even in the later stages of pregnancy, without accident, just as other abdominal operations are often feasible; but any pancreatic resection to the point of diabetes seems absolutely incompatible with continuance of gestation. The possibility that this disturbance may be due to the sudden deficit of the internal pancreatic function was excluded by two sets of controls; first, the results are just as bad when the entire uncinate process is left as a subcutaneous graft in addition to the usual pancreatic remnant; second, pregnant dogs ordinarily survive a week of fasting and phlorizination without abortion, though the glycosuria and acidosis apparently represent a greater disturbance of carbohydrate metabolism than that following the pancreatic operations mentioned.

An attempt to test the influence of the increased food requirement and lactose formation of the lactation period was made as follows.

Dog C3–90, mongrel, in excellent condition, weighing 20.5 kilos, was found pregnant in an operation on May 25, 1916, when the splenic process and body of the pancreas down to the main duct were removed, with the idea that diabetes might later be produced by a very easy operation for removal of the uncinate process. The pregnancy continued uneventfully, but by mistake too long an interval was allowed, and on June 22 the dog gave birth to eight healthy puppies. She was an excellent mother and the puppies all thrived until, on June 29, the uncinate process was removed in an operation requiring only a few minutes. There was quick recovery from the brief etherization, and the dog showed the usual devotion and nursed the puppies immediately on being returned to the cage. She acted well and lively but ate very little on the following days, during which time the pups continued to nurse though the mother paid less attention to them. By July 4 her appetite was fully restored, but she refused to have anything further to do with the pups, and even injured them if they approached to nurse. Part of the trouble may have been due to tenderness about the abdominal wound, but there seemed also to be a genuine breaking up of the physiology and psychology of lactation by the operation. Milk rapidly disappeared from the breasts. Diabetes remained absent notwithstanding glucose feeding. Whether the disturbances observed were peculiar to the pancreatic operation or might follow any other abdominal interference, the experience showed that this method was not applicable for studying the influence of lactation upon diabetes.

Numerous microscopic examinations have been made of the pancreas of dogs in various stages of pregnancy and of a few during lactation, without the finding of any departure from the normal in any respect.

It was evident that a satisfactory study was possible only through the occurrence of pregnancy in animals already diabetic, so as to reproduce exactly the conditions encountered in diabetic women. Properly prepared animals with potential diabetes are normal in their entire behavior, and only the unfavorable laboratory conditions made the experiment difficult. It was hoped that cats might be particularly suitable for the purpose, but in the only instance in which a partially depancreatized cat became pregnant the experiment ended in failure, as follows.

Cat A1–84. Female; strong adult; weight 3.5 kilos. December 20, 1913, pancreatic tissue was removed, not quite to the point of producing diabetes. The cat remained continuously free from glycosuria on meat, bread and milk diets thereafter, but had a permanently lowered tolerance, as shown by the production of glycosuria in subcutaneous glucose tests by doses between 1 and 1.5 grams per kilo. Impregnation occurred February 18, 1914. Thereafter the diet was meat and milk, mixed with as much lactose as the animal could be induced to take. Considerable carbohydrate could thus be given, though cats usually object strongly to the sweetness of glucose or saccharose. About the middle of March the animal was noticed to be weak and unwell. Abortion occurred March 16 and death March 18. There was no diabetes and the pancreas remnant was normal. The fatal outcome was almost certainly a sugar intoxication (4), with no specific relation to the partial pancreatectomy or to diabetes. Cats may possibly prove to be well suited to pregnancy experiments in diabetes, but they are as a rule an unfavorable species for carbohydrate over-feeding.

Far more numerous attempts were made with partially depancreatized dogs during three years, but all failed owing to the unfavorable environment. Finally a successful experiment became possible in dog B2-00, which was particularly valuable for the purpose because of the long previous records which had established the tolerance accurately. These observations have been reported in previous papers, especially no. 3 of series I (5) and no. 1 of series II (6).

A series of operations beginning in 1913 had made the dog nearly diabetic, but after the removal of an additional 0.8 gram of pancreatic tissue on September 6, 1916, it was still impossible to maintain glycosuria with the heaviest bread and glucose feeding. Pregnancy then became evident, though the exact time of its beginning was unknown. Tests of the tolerance were performed by feeding, as described below. As diabetes remained absent with advancing pregnancy, there was danger that the entire result would be negative.

Accordingly, on December 16, 1916, 0.1 gram pancreatic tissue was removed, in an operation so short and easy that the dog did not even lose appetite for the day. Diabetes resulted, as proved by the fact that plain bread and soup feeding now maintained slight glycosuria (0.4 to 0.8 per cent, in 400 to 680 cc. urine). December 26 on this diet the plasma sugar was 0.091 per cent before feeding, 0.182 per cent four hours after. The special feeding test was repeated in late pregnancy on December 30.

As an additional experiment, it was attempted to learn whether the pregnant dog with latent diabetes had any special tendency to acidosis. Therefore nothing was fed on December 31, and only 300 grams suet daily on January 1, 2 and 3 (1917). Abortion then occurred, though the dog appeared only slightly unwell and remained free from acetone bodies in urine and blood, both before and afterward. The plasma sugar was constantly normal (0.089 to 0.105 per cent) after omission of carbohydrate, both before and after abortion. The carbohydrate tests had seemed harmless, and it may be possible that the fat feeding was responsible for the abortion even without acidosis.

January 27, the special feeding test was repeated as a control in the non-pregnant condition. The existence of diabetes was unmistakable. The diet was then changed to meat to prevent downward progress, and the dog was left most of the

Dog B2–00. Tests with feeding 100 grams beef lung, 200 grams bread and 150 grams glucose

DATE	WEIGHT	PLASMA SUGAR	Hb.	URINE		REMARKS
				Volume	Glucose	
1916	*kgm.*	*per cent*	*per cent*	*cc.*	*per cent*	
November 21	13.4	0.106	101	18	0	Before feeding
		0.123	97	16	0	2 hours after feeding
		0.156	98	24	Faint	4 hours after feeding
		0.128	96	38	Faint	6 hours after feeding
November 23		0.092	95		0	Before feeding same diet as Nov. 21
		0.095	88	13	Faint	2 hours after feeding
		0.139	92	No	urine	4 hours after feeding
		0.130	93	105	Faint	6 hours after feeding
December 16	Removed 0.1 gm. pancreatic tissue. Dog pregnant					
December 30	15.6	0.085			0	Before feeding
		0.345		212	3.45	2 hours after feeding
		0.455		192	6.46	4 hours after feeding
		0.500		68	5.89	6 hours after feeding
1917						
January 7	12.9	0.125		7	0	Before feeding. After abortion
		0.400		25	7.13	2 hours after feeding
		0.400		35	6.68	4 hours after feeding
		0.333		33	8.00	6 hours after feeding
February 23	16.3	0.116			0	Before feeding
		0.238		18	0.63	2 hours after feeding
		0.244		17	0.57	4 hours after feeding
		0.232		22	0.69	6 hours after feeding
May 17	15.6	0.130			0	Before feeding. (Early pregnancy)
		0.346		39	4.16	2 hours after feeding
		0.435		73	7.32	4 hours after feeding
		0.384		44	4.82	6 hours after feeding
July 11	14.9	0.106			0	Before feeding. (Late pregnancy.)
		0.322		35	4.72	2 hours after feeding
		0.304		65	3.95	4 hours after feeding
		0.312		68	5.58[1]	6 hours after feeding
July 19	13.0	0.077		0	0	Before feeding. (After delivery)
		0.200		23	0.72	2 hours after feeding
		0.218		16	0.92	4 hours after feeding
		0.232		13	0.95	6 hours after feeding

DATE	WEIGHT	PLASMA SUGAR	Hb.	URINE		REMARKS
				Volume	Glucose	
1917	*kgm.*	*per cent*	*per cent*	*cc.*	*per cent*	
August 9	12.9	0.081			0	Before feeding
		0.164		18	Very faint	2 hours after feeding
		0.109		36	Faint	4 hours after feeding
		0.135		17	Faint	6 hours after feeding
October 5	13.5	0.109			0	Before feeding
		0.159		17	0	2 hours after feeding
		0.145		36	Faint	4 hours after feeding
		0 152		46	0.31	6 hours after feeding
November 22	14.0	0.109			0	Before feeding
		0.204		24	Faint	2 hours after feeding
		0.238		36	0.39	4 hours after feeding
		0.125		24	0.44	6 hours after feeding

time with a male in the hope of a second pregnancy. The plasma sugar meanwhile remained normal (0.087 to 0.113 per cent) on the carbohydrate-free diet. The special feeding test was repeated on February 23 and May 17.

Pregnancy then became manifest, and the feeding test was repeated in this condition on July 11. June 28 and July 2, the plasma sugar on the meat diet was normal (between 0.075 per cent before feeding and 0.105 per cent after feeding).

July 12, less than 0.1 gram of pancreatic tissue was removed without appreciable disturbance.

Three pups were born on July 16. They were probably 2 or 3 days premature, and were all dead by July 19 owing to failure to nurse properly. This result may have been independent of the experimental conditions, for the dog was of a type which often has troubles with puppies.

The special feeding test was repeated on August 9, October 5 and November 22.

Owing to the perfect manner in which the dog bore the experiments through two pregnancies, the questions at issue received very satisfactory answers as follows:

a. In the first instance, pregnancy failed to produce diabetes in an animal which was so close to the verge that diabetes resulted from the removal of 0.1 gram additional pancreatic tissue. Against an assumption that the diabetes here was due largely to the pregnancy and only partially to the pancreas operation is the fact that diabetes persisted after termination of the pregnancy. Also in the second instance, when the dog was known to have mild diabetes but was free from symptoms on meat diet, pregnancy failed to produce either glycosuria or hyperglycemia on this diet.

b. The removal of a bit of pancreatic tissue in the latter part of each pregnancy supplemented the observations of the negative effects of gestation and especially demonstrated the absence of vacuolation of the islands first when the animal was not quite diabetic and second when she was mildly diabetic. The absence of such hydropic changes with latent diabetes corresponds fully to the experience in non-pregnant animals.

c. The passage of any appreciable quantity of pancreatic internal secretion from the fetuses to the mother is disproved by two facts. First, the occurrence of diabetes following the operation of December 16, 1916, was not prevented; in other words, there was no transfer of pancreatic hormone sufficient to compensate for the loss of 0.1 gram of maternal pancreatic tissue. Second, the feeding tests demonstrated an actual aggravation of the diabetes during pregnancy. Test meals were used instead of intravenous glucose injections because according to previous experience (7) they afford a more accurate index of the diabetic condition and also because they seemed to promise less danger of abortion or other accidents. Attention may be called to the results of these tests as shown in the table.

The test meal consisted of 100 grams beef lung, 200 grams bread and 150 grams glucose. Only slight hyperglycemia and faint glycosuria resulted from this diet on November 21 and 23, when the dog was non-diabetic. The removal of 0.1 gram pancreatic tissue on December 16 produced a radical change, so that the hyperglycemia and glycosuria in the test of December 30 were of plainly diabetic character. The tolerance was fully as low on January 7, after termination of pregnancy, but the dog was still unwell from the recent abortion. The test of February 23 showed a considerably better tolerance as judged by both blood and urine.

May 17, early in the second pregnancy, the test showed a well-marked fall in the assimilation, and the result was not greatly different in the late stage of pregnancy on July 11. The bit of pancreas removed on July 12 was so tiny that it apparently had little effect upon the tolerance. At any rate, in spite of this operation, the assimilation on July 19, three days after normal delivery, showed a decided improvement. This improvement was still greater on August 9, after all puerperal disturbance had subsided. It was also maintained up to October 5, the length of the experiment and the uniformity of results being thus sufficient to exclude accidental fluctuations of tolerance.

CONCLUSIONS

1. No positive influence of the sex glands upon diabetes was demonstrable by extirpation experiments. Also no anatomic changes in the pancreas were perceptible with pregnancy or lactation.

2. Observations upon a partially depancreatized dog during pregnancy are opposed to the view that any appreciable quantity of internal pancreatic secretion passes from the fetus to the mother.

3. A distinct lowering of carbohydrate assimilation was shown during pregnancy. This was not clearly associated with the increase of metabolism, in the sense either of increased food requirement or new tissue formation, for it seemed approximately the same in early and late pregnancy and was also evident during the illness following abortion. It may therefore be regarded chiefly as a toxic manifestation and thus classifiable with the influence of infection. The effect is relatively slight, because pregnancy failed to produce diabetes in a dog where the removal of 0.1 gram pancreatic tissue sufficed for the purpose, and also after the dog was demonstrably diabetic on carbohydrate diet pregnancy gave rise to neither glycosuria nor hyperglycemia on carbohydrate-free diet.

The tests with partial pancreatectomy, which affords the most exact method of study, suggest that Carlson's results in totally depancreatized dogs are to be interpreted as cachexia. Clearer information of the influence of pregnancy upon the internal pancreatic function is also afforded by the freedom from the variables which enter into clinical cases. The slight tendency to aggravation of the diabetes and the ready control by diet support Joslin's experience of the feasibility of completion of pregnancy by diabetic women under suitable conditions of treatment. If the toxic factor is the principal one, as suggested, the possibility remains that the injurious action in some women may be considerably greater than in dogs and accordingly may require more radical measures.

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RHYTHMICITY OF THE PYLORIC SPHINCTER

HOMER WHEELON AND J. EARL THOMAS

From the Department of Physiology of the St. Louis University School of Medicine, St. Louis, Missouri

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The functions of the pyloric sphincter are usually considered dependent upon the physical condition and relative degree of acidity of the gastric content. Such an explanation fails in great part to account for certain phenomena associated with the processes of gastric evacuation both in health and disease. It also fails to consider the purpose of gastric motility save for mixing the gastric contents and discharging them into the duodenum at irregular periods of opening of the sphincter. However, recent work tends to show that the opening and closing of the sphincter is related, not only to the degree of fluidity and acidity of the gastric content, but also to peristalsis and the degree of tonicity demonstrated in the stomach.

Our present interest in the physiology of the sphincter was stimulated by our inability to explain, upon accepted theories, radiographic observations made upon the human stomach. We therefore undertook to study, upon laboratory animals under easily controllable conditions, the motility of the pyloric sphincter. In this study the term pyloric sphincter means that narrow band of muscle constituting the last portion of the stomach.

METHODS

Forty-four experiments were performed upon dogs in this series. All operative procedures were carried out under ether anesthesia. Unanesthetized dogs were studied radiographically. In 14 experiments records were obtained immediately following operation and while the animal was under the influence of a light anesthesia. Six dogs were studied from 5 to 18 hours after operation, no anesthesia being used during the taking of records. One animal was studied 3 days after operation, another after an interval of 2 weeks. Four animals were operated under ether and morphine (10 mgm. per kilo) and studied at once, ether being discontinued during the observations. This procedure gave a quiet animal with a very active stomach. Although the type of sphincter action under this anesthesia was similar to that obtained under other anesthesias, it was discontinued because of the known action of morphine upon the gastro-intestinal tract. The most constant graphic results were obtained with only sufficient ether to abolish voluntary movements. Under such an anesthesia the behavior of the sphincter is more constant and uniform than in the unanesthetized animal under experimental conditions.

The operations in most cases consisted in opening the abdomen by a midline incision, entering the fundic portion of the stomach and sewing the recording apparatus in place. The gastric incision was then closed with a pursestring suture about the rubber tube leading from the anchored balloon. The opening in the abdomen was closed with a running suture. Aseptic precautions were observed in all cases in which the animal was permitted to survive 12 hours or longer. In 3 animals the muscle on either side of the pyloric sphincter was cut, thus confining action upon the apparatus to the sphincter proper.

All apparatus except the recording instruments was designed and constructed especially for this work. The apparatus is the equivalent of a balloon and is so constructed that it can be placed and maintained within the lumen of the pyloric sphincter. These balloons, or as we have termed them, *pylorographs*, are of three types: *a*, flexible, closed pylorograph; *b*, rigid, closed pylorograph, and *c*, open, flexible pylorograph.

The closed flexible pylorograph (fig. 1, *a*) consists of a short section of finger cot stretched over two cones of hard rubber the apices of which face each other. One cone is perforated by a small hole which leads out through a nipple on its base for the attachment of a rubber tube. When in position the rubber is pressed down upon the sloping surfaces of the cones by the tonus of the sphincter to such an extent that the recording apparatus is affected by the contraction of a width of muscle not exceeding 5 mm. This device when placed in position tends to remain there because of the V-shaped surfaces offered to the pyloric ring. Complete fixation of the pylorograph was obtained by passing a ligature around the base of each cone and securing it in the muscle.

The rigid pylorograph is similar to the one described above save that the two cones remain connected (fig. 1, *b*).

The open, flexible pylorograph (fig. 1, *c*) is constructed on the same principle as the flexible, closed pylorograph except that an open tunnel 4 mm. in diameter runs throughout its entire length. The apices of the two cones are connected by means of a piece of rubberdam tubing.

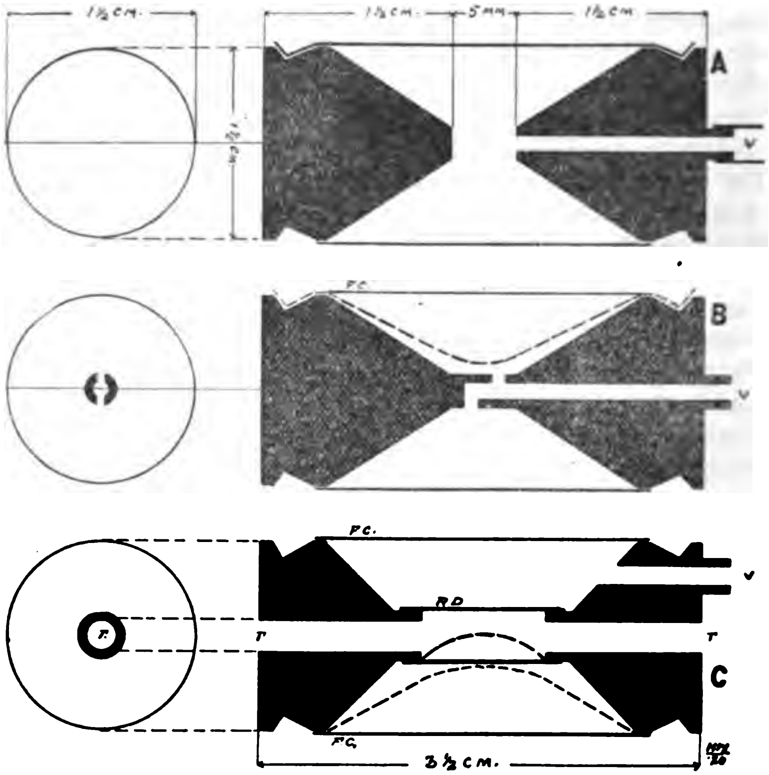

Fig. 1. Illustrations of the various types of pylorographs. *A*, Closed flexible pylorograph. *B*, Closed rigid pylorograph. *C*, Open flexible pylorograph. *V* Air transmission vent for tube connection with water manometer. *F.C.*, Flexible wall of pylorograph made of finger cot. *T.*, Opening connecting stomach with duodenum. *R.D.*, Tube of rubber dam composing inner wall of air chamber. Dotted lines indicate positions of the rubber surfaces during contraction of the sphincter.

The bases are connected by a section of finger cot. This device, the equivalent of a tunnelled cylindrical balloon, permits the sphincter to demonstrate its motility and at the same time permits material to leave the stomach.

Graphic records were obtained with tambours, and piston and bellows recorders. The pylorograph was connected with the recording apparatus through a water manometer. The manometer was adjusted to a pressure (3 to 30 cm.) which gave best results in any given case. Air transmission was used throughout.

RESULTS

The pyloric sphincter demonstrates two types of motility; *a*, active rhythmic contractions (figs. 2 and 3), and *b*, tone waves (figs. 5 and 6). The characteristics of these two types of action are as follows.

a. Rhythmic contractions. The rhythmic movements of the sphincter are characterized by contractions and relaxations each followed by a quiescent period or pause (fig. 3). These contractions occur at the rate of from 3 to 5 per minute; that is, each cycle is completed in an interval of from 12 to 20 seconds. The phase of contraction is 4 to 5 seconds, the phase of relaxation 3 to 7 seconds; the quiescent phase plus the period of inhibition prior to contraction occupy the remainder of the cycle. These figures are necessarily only relative because of the different rates of contraction shown by various animals, the depth of the anesthesia, and also because of the general motility of the stomach and small intestines. During periods of rhythmic action of the sphincter the contractions and relaxations are uniform in degree; that is, the movements are initiated and consummated from a constant level (figs. 2 and 3). There is usually a definite degree of relaxation (inhibition) of tone immediately preceding a contraction. At times relaxation continues from the completion of contraction until the beginning of a second contraction, no definite phase of quiesence or inhibition being shown. Similar results were obtained both from the filled and recently emptied stomach.

The cycles of the sphincter are definitely altered under various conditions; also, periods of increased activity may occur; however, as will be shown in a later communication, such alterations bear a definite relation to anesthetics, trauma and the activities of the gastro-intestinal canal.

Rhythmicity of the sphincter is not lost following denervation of the stomach (fig 4).

b. Tonicity of the sphincter. The tonicity of the pyloric sphincter is gained or lost during a series of rhythmic contractions of which the heights of the individual contractions vary in proportion to the degree

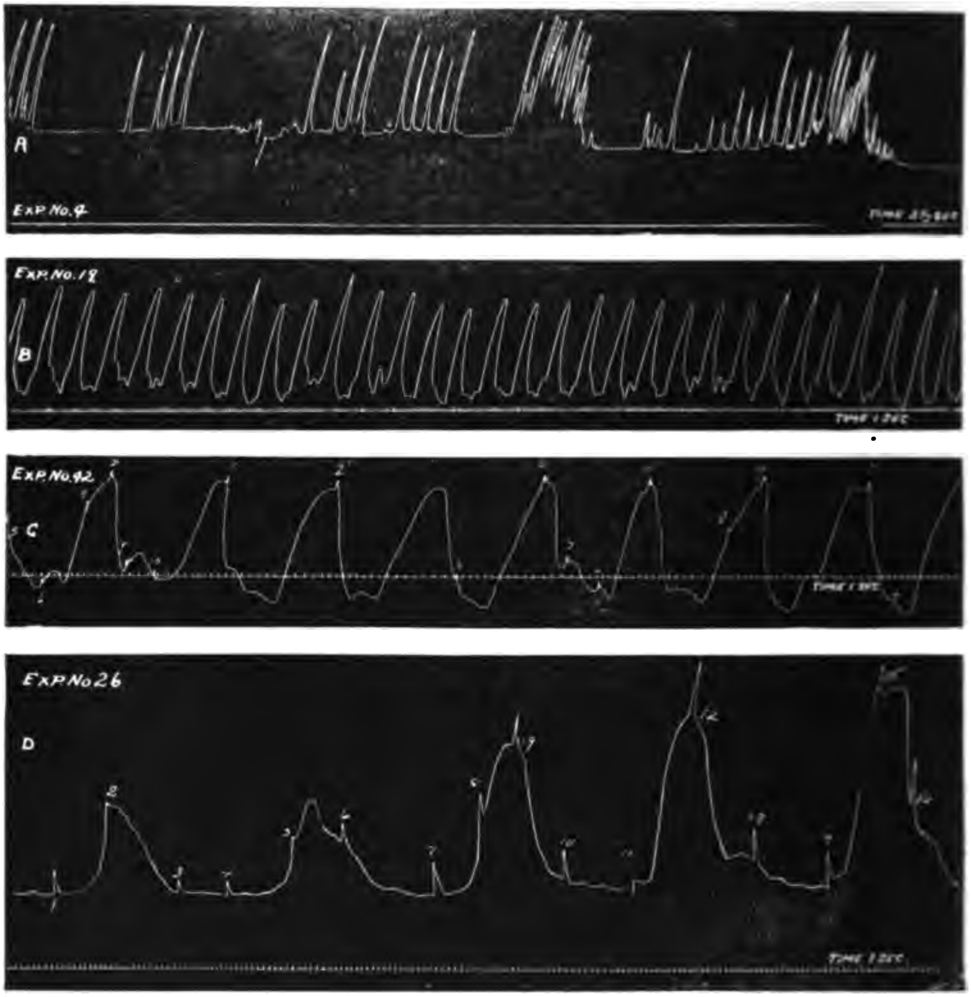

Fig. 2. Pylorograms obtained from four different experiments; *A*, experiment 4; *B*, experiment 19; *C*, experiment 42; *D*, experiment 26. Animals under ether anesthesia. Graph *A* obtained with tambour, *B* and *C* with piston recorder, *D* with bellows recorder. Time, 2½ seconds in trace *A*, 1 second in *B*, *C* and *D*.

Trace *A* shows periods of inactivity followed by rhythmic contractions and alterations in tonicity. Traces *B*, *C* and *D* demonstrate periods of constant rhythmic activity. Note the variation in the height of contractions in traces *A* and *D*. The four graphs are typical of the entire series of experiments.

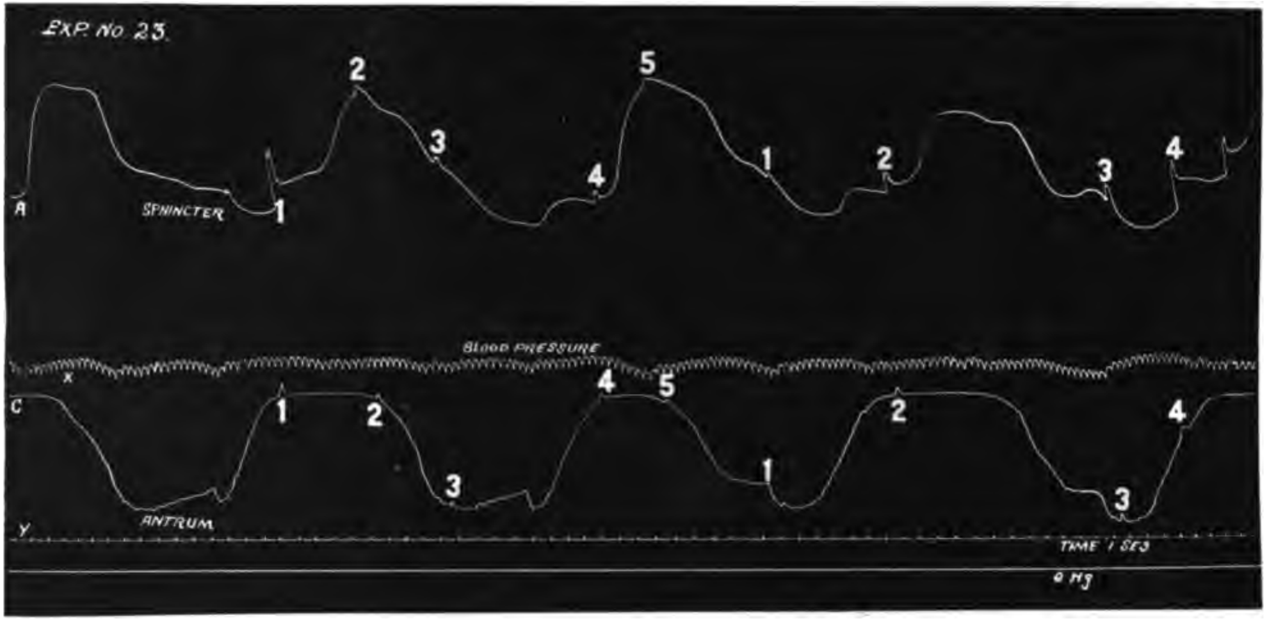

Fig. 3. Experiment 23; February 7 1920. Ether used throughout experiment. Records begun immediately following operation. Double rigid enterograph used. *A*, Sphincteric contractions. *X*, Blood pressure tracing *C*, Antral contractions. *Y*, Time in seconds. Numerals denote synchronous points.

Note that the antrum has reached its maximum at the beginning of the sphincter's contraction, the latter reaching its greatest degree of contraction just as the antrum begins to relax.

of tonicity gained (figs. 5 and 6). On the other hand, periods of increased or decreased rhythmic contractions may appear at a time of constant tonicity (fig. 5, trace *C*). Waves of increased tonicity appear to result because of a lengthening in the time required for complete relaxation; that is, a second wave of contraction appears during the relaxation phase of the previous cycle. A reduction in the degree of tonicity accompanies an increased degree of lengthening of the relaxation phase of the rhythmic cycles, the rate of the rhythmic contractions remaining constant.

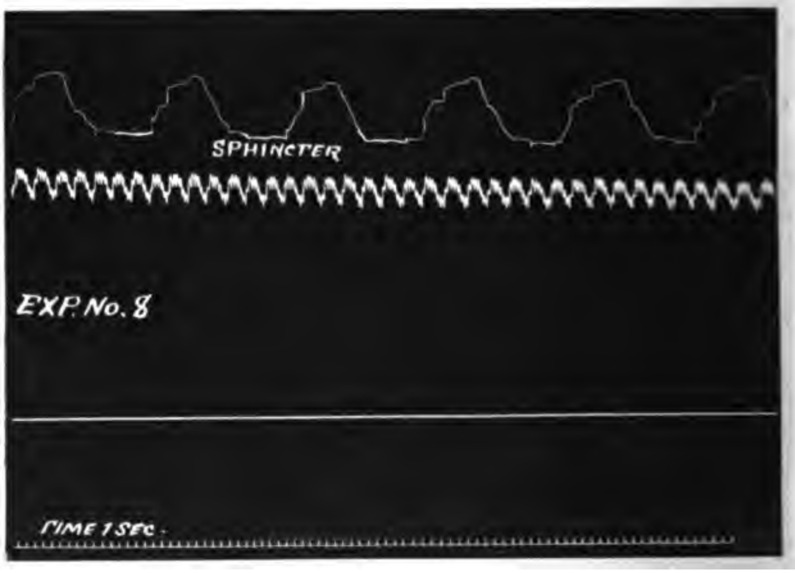

Fig. 4. Experiment 8; January 17, 1920. Rhythmic contractions of the pyloric sphincter following denervation of the stomach. Ether anesthesia. Time in seconds.

The rhythmicity and type of sphincter action are not altered by increasing or decreasing, within limits, the pressure within the pylorograph. However, if the pressure is greatly increased the height of the individual contractions is decreased. Longer excursions of the writing lever are recorded when the pressure in the pylorograph is low (3 to 10 cm. of water). In other words, the greater the degree of resistance offered by the pylorograph to the force of the sphincter's action the smaller becomes the excursion of the muscle while acting. The primary effect of distention of the balloon in the pyloric canal is to excite the sphincter to rhythmic action. Following this there usually appears a gradual loss in tone for several moments after which the rhythmic contractions appear from a constant level. The rapidity with which the sphincter adapts its tonicity to an alteration in resistance is remarkable. Apparently, the normal tonicity is only sufficient to close the sphincter or to approximate its surfaces against those of a body in its lumen. Non-resisting materials permit of complete occlusion of the lumen during the positive phase of the sphincteric cycle. Tonicity

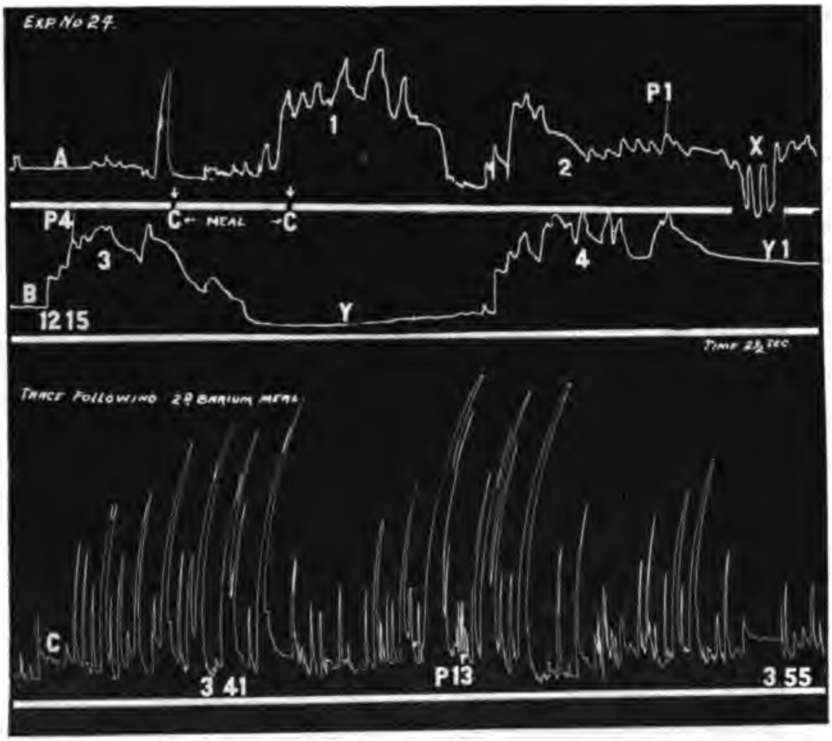

Fig. 5. Experiment 24; February 9, 1920. Pylorograms obtained by use of open pylorograph. Animal operated under ether. Records begun 18 hours later. Piston recorder used for registration. Experiment checked by radiographic records. *A*, *B* and *C*, Sphincter action. *C–C*, Administration of 8 ounces of barium mixture by stomach tube. *1*, *2*, *3* and *4*, Tone waves carrying contraction waves. Trace *C* shows three active and four reduced phases of activity.

of the sphincter appears to be unusually high immediately following the ingestion of food, either normally or by means of the stomach tube. Tonicity is also high immediately following the opening of the abdominal cavity. Doubtless this is reflexly the result of peritoneal irritation. Tonicity is not lost by reason of operative procedures on the stomach.

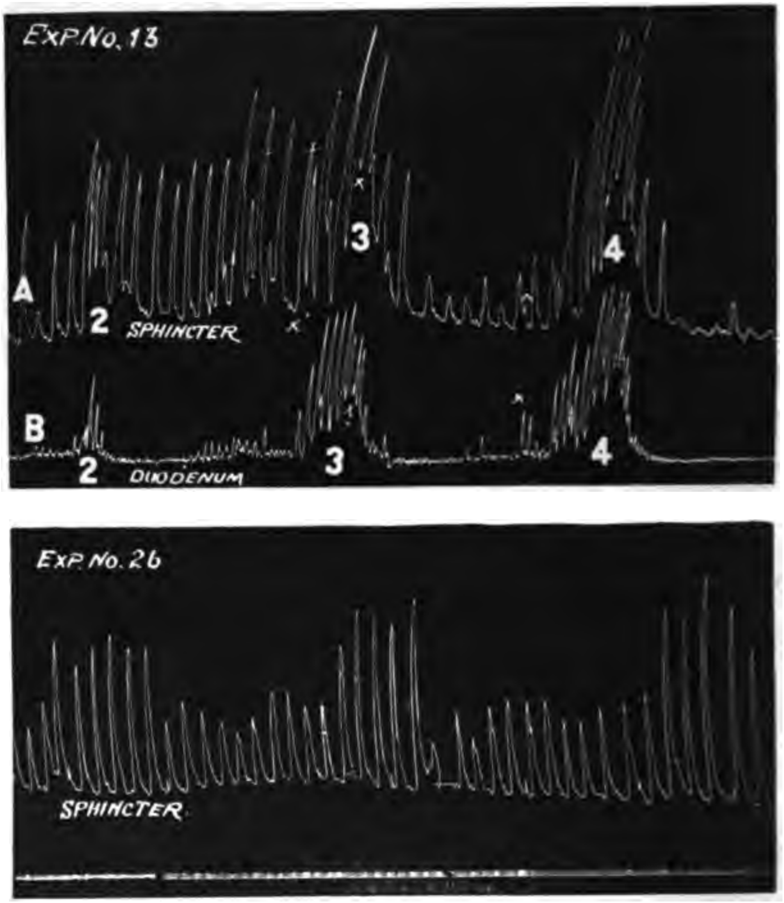

Fig. 6. Experiment 13; January 9, 1920. Ether anesthesia for operation. Double flexible enterograph. Records obtained 3 days after operation upon the conscious animal. *A*, Sphincter contractions. *B*, Duodenal contractions. Note periods of heightened tone of the sphincter at times of pronounced activity on the part of the duodenum (*3* and *4*). *X*, Synchronous points. Time in seconds.

The approach of the pylorograph or examining finger to the antral region causes marked contraction of the pars pylorica. Only under the influence of a deep or surgical anesthesia does the sphincter lose tone completely.

SPECIAL EXPERIMENTS

In dog 25 in which a permanent gastric fistula had been established, it was possible to insert the finger into the antrum and even through the pyloric canal into the duodenum without placing the animal under ether or causing it much discomfort. The primary effect of the finger in the antrum was to excite a state of violent and maintained contraction. Under a constantly applied pressure contracture passed off and the finger was finally permitted to pass through the sphincteric canal. With the finger in the canal one could distinctly feel pressure applied rhythmically during the positive phases of the sphincteric cycles. The sphincter never relaxed completely nor drew away from the finger during the relaxation phase or the period of quiescence. The observation that an initial irritation of the antrum causes a heightened degree of tonicity in the pars pylorica corroborates the generally accepted views as to the functions of these parts in relation to solid objects in the stomach.

In an effort to throw more light on the function of the sphincter the following experiment was performed. An open pylorograph (fig. 1, *c*), as described above, was anchored in the pyloric canal. Graphic records were begun 18 hours following operation (fig. 5). At this time the sphincter demonstrated powerful contractions which occurred irregularly. The animal was then placed on the radiographic table and given an 8-ounce meal of barium sulphate and milk by means of the stomach tube. Immediately following the withdrawal of the tube a marked tone wave appeared on the graphic tracing, superimposed upon which was a series of contractions of varying degree (fig. 5, *A-1*). This was followed by a series of smaller waves each of which lasted from 3 to 7 minutes. Four such tone waves are shown in tracings *A* and *B* of the same figure. As previously stated, these tone waves are built up during a series of rhythmic contractions and lost through a series of gradual and prolonged relaxations. At *X* in figure 5 a series of relaxations permitted the recording lever to drop below the tone level held by the sphincter prior to the administration of the meal. At points marked *Y* and *Y-1* there is a total absence of rhythmic contraction although the tonicity of the sphincter at *Y-1* is markedly above that at *Y* where the level is lower than the pre-meal normal. A radiogram taken at the height of a rhythmic contraction (*P.1*), and at a time of relatively high tonicity, showed the antrum in a state of high tonicity with the pyloric canal closed. As time went on these severe tonic contractions gradually gave way to a constant tone level and rhythmic contractions of varying height (fig. 5, *c*). The stomach emptied itself of the barium mixture in less than 80 minutes. At the end of this time a second meal was given. Rapid distention of the operated stomach easily produces vomiting. This difficulty was overcome by using a small catheter as a stomach tube. The type of sphincter action shown in tracing *C* was continued for 4 hours, at the end of which time the stomach was completely free from the second meal.

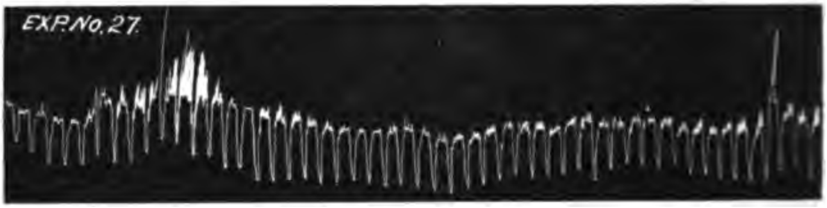

Fig. 7. Experiment 27; February 19, 1920. Ether used throughout experiment. Records begun immediately following operative procedures. Tambour myograph and piston recorder used to obtain graph. Because of construction of the myograph the contraction phase is recorded as the downstroke; relaxation the upstroke. Note the regularity of contraction and the tone level changes.

This experiment seems of special interest because it demonstrates that a foreign body in the pyloric canal through which the gastric contents may pass does not alter the normal progress of gastric evacuation. Further, it supplies information from both the experimental and the radiographic standpoint which indicates that the sphincter is rhythmical in its activities. The development of tone waves in this case does not differ in character from those observed by other types of recording apparatus through which the gastric contents may not pass.

A third type of experiment was performed to check the graphs obtained by means of the closed and open types of pylorographs. In these experiments, three in number, a tambour myograph was attached to the exterior of the sphincter, the animal being immersed in a tank of warm saline solution. The type of rhythmic contractions and tone waves shown in figure 7 are common to the three experiments, and they show no fundamental variation from tracings obtained with other types of apparatus. The animal (no. 27) from which this figure was obtained had been fed about an hour before the experiment started. Often a gurgling noise was audible as material was forced from the antrum into the duodenum, these occurring during a wave of contraction of the pars pylorica.

The results obtained from the various types of experiments and radiographic studies are similar to each other, therefore the movements described for the pyloric sphincter may be considered as representative of normal functioning of this organ.

DISCUSSION

The closure of the pyloric sphincter, according to the prevailing theories, results because of: *a*, the presence of solid masses in the antrum which mechanically excite the mucosa of this region; *b*, an insufficient acidification of the gastric content; and *c*, the presence of acid chyme in the duodenum. Following liquefaction and acidification of the gastric contents these mechanical and chemical stimulants cease and the sphincter relaxes to permit the ejection of chyme into the duodenum.

The theory of an "acid control of the pylorus" (Cannon) does not account for the rapid discharge of water and solutions of neutral egg white from the stomach, neither does it explain the rapid clearance of the stomach in certain pathological conditions. In part the theory of fluidity accounts for the rapid evacuation of the stomach following the ingestion of fluid masses. However, this does not explain the processes involved in the control of the sphincter. Granting that the two theories do explain a certain number of facts relative to the control of the pylorus, we still have to account for pyloric activity when functioning in the absence of acid. That is, something more than fluidity and acid is necessary to open the sphincter to permit the passage of material into the duodenum. Granting that the sphincter is open, all theories agree that peristaltic contractions in the stomach are directly responsible for the discharge of gastric contents. Hence it may be assumed that intragastric pressure and peristalsis bear some definite relation to the activities of the sphincter. That posture and peristalsis do act in such a manner as to facilitate the emptying of the stomach has been pretty definitely shown.

Neilson and Lipsitz (5) have shown that posture to a great extent determines the time of retention of water in the stomach. Individuals lying on their right side retain less of a given amount of water at the end of a stipulated time than individuals assuming other positions. Cole (2), from a study of serial radiograms of the human stomach, has shown that the activities of the sphincter bear a definite relationship to the activities of the antrum in that the amount of contraction of the sphincter is in proportion to the activity of the gastric waves. He has also shown that during the active phase (contraction) of every gastric cycle the pyloric ring is open and a small portion of the gastric contents is propelled through its lumen into the reservoir cap. The terminal wave (peristaltic) which has meanwhile been advancing toward the sphincter, upon attaining it, effects its closure. The recent article by Luckhardt, Phillips and Carlson (4) clearly demonstrates that both in man and dogs the pyloric sphincter opens for the ejection of chyme with the arrival at the sphincter of powerful advancing rings of contraction and a general increase in the tone of the musculature of the stomach as a whole. Their observations demonstrate that a more definite relation exists between the muscular activity and the opening of the sphincter than between the opening of the sphincter and the reaction of the gastric contents. Ivy (3) made the suggestion prior to the appearance of the paper by Luckhardt, Phillips and Carlson, that the rhythmic discharge of water from the dog's stomach is such that it could very well correspond to peristaltic activity.

Such observations clearly demonstrate that the functions of the pyloric sphincter are dependent, in part at least, upon gastric motility. Our observations also tend to show that the sphincter because of its rhythmic type of motility acts in such a manner as to supplement gastric motility. Further observations to be reported later also show that the rhythmic contractions of the sphincter bear a definite and constant relation to the motility of the stomach (fig. 3).

SUMMARY AND CONCLUSION

1. A method is described for recording the motility of the pyloric sphincter.
2. The pyloric sphincter of the dog demonstrates rhythmic activity or cycles of motility which occur at the rate of from 3 to 5 per minute.
3. A single cycle of motility is characterized by a phase of contraction, relaxation and quiescence followed by a definite phase of inhibition prior to a subsequent contraction.

4. The sphincter demonstrates tone changes, such changes being gained or lost because of shortening or lengthening of the relaxation phase of the rhythmic cycles.

5. The observations here reported, therefore, show that the pyloric sphincter of the dog possesses the property of rhythmic contractility, the degree of which is influenced because of changes in tonicity.

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A DIFFERENCE BETWEEN THE MECHANISM OF HYPERGLYCEMIA PRODUCTION BY ETHER AND BY CHLOROFORM

ELLISON L. ROSS and L. H. DAVIS

From the Department of Physiology and Pharmacology, Northwestern University Medical School

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It has already been reported that there are grounds for believing that the hyperglycemia produced by ether anesthesia is due chiefly to the action of ether to reduce the influence of the internal secretion of the pancreas (1). It is considered that this reduction of the action of the internal secretion of the pancreas is a reduction of the inhibitor influence on glycolysis. Since the primary source of blood dextrose is liver glycogen, any injury to the liver should affect the ease with which glycogen is set free. Whether the injury to the liver cells should make the liberation of dextrose easier or more difficult there is no way to foretell. It is well known that chloroform is capable of producing liver pathology. Davis and Whipple (2) showed that chloroform anesthesia produced injury to liver cells and this injury could be increased by fasting before the administration of chloroform. It was thought worth while to compare the hyperglycemia from ether and from chloroform with special reference to the injury produced in the liver.

Experimental work. A group of five normal undieted dogs was given ether for half an hour. Anesthesia was induced by inserting the animal's head into a cylinder into which air that had passed through ether was forced. The animals were bled before anesthesia and after fifteen minutes of anesthesia. The blood sugar was determined by Benedict's method (3). The next day the same procedure was repeated except that the animals were kept under the anesthetic only fifteen minutes. The results are given in tables 1 and 2.

A second group of dogs was given chloroform to the surgical anesthetic stage and kept at this degree of anesthesia for half an hour and the following day the response to fifteen minutes of ether anesthesia was determined. The bleeding, analyses and administration of the anesthetic were carried out as before. The results are given in table 3.

TABLE 1

Ether hyperglycemia of a group of normal dogs

ANIMAL	GLYCEMIA BEFORE ETHER	GLYCEMIA AFTER 15 MINUTES ETHER ANESTHESIA	INCREASE
1	0.113	0.143	0.030
2	0.084	0.111	0.027
3	0.116	0.127	0.011
4	0.096	0.141	0.045
5	0.105	0.132	0.027
Average..........	0.1028	0.1308	0.028

TABLE 2

Ether hyperglycemia of same group of dogs, day following half an hour ether

ANIMAL	GLYCEMIA BEFORE ETHER	GLYCEMIA AFTER 15 MINUTES ETHER ANESTHESIA	INCREASE
1	0.100	0.166	0.066
2	0.093	0.106	0.013
3	0.117	0.140	0.023
4	0.097	0.129	0.032
5	0.085	0.144	0.049
Average..........	0.1004	0.1370	0.0366

TABLE 3

Ether hyperglycemia one day following half hour chloroform anesthesia

ANIMAL	GLYCEMIA BEFORE ETHER	GLYCEMIA AFTER 15 MINUTES ETHER ANESTHESIA	INCREASE
6	0.095	0.106	0.011
7	0.092	0.116	0.024
8	0.099	0.120	0.021
9	0.097	0.136	0.039
10	0.097	0.106	0.009
Average..........	0.0960	0.1168	0.0208

TABLE 4

Ether hyperglycemia following half hour chloroform anesthesia after fast

ANIMAL	GLYCEMIA BEFORE ETHER	GLYCEMIA AFTER 15 MINUTES ETHER ANESTHESIA	INCREASE
11	0.094	0.097	0.003
12	0.093	0.134	0.041
13	0.088	0.094	0.006
14	0.094	0.116	0.022
15	0.095	0.115	0.020
Average..........	0.0928	0.1112	0.0184

A third group of animals fasted two days. Then these dogs were put through the same treatment as the second group. The results are given in table 4.

Discussion. When a drug is administered repeatedly the later reactions usually differ in degree from the first one. This may be due to decreased or increased sensitiveness of nerves, glands or tissue, or injury to cells. To determine whether or not ether had less power to produce hyperglycemia the second day than the first, the first two series of tests were made.

According to tables 1 and 2, the average glycemia before ether the first day was 0.1028 and the second day 0.1004 per cent. This small difference is negligible. Half an hour of ether anesthesia did not change the amount of blood sugar found the following day. This harmonizes with the general impression that ether anesthesia causes very little if any injury to the subject. The first day fifteen minutes of ether anesthesia brought the glycemia up to 0.1308 per cent and the following day the same procedure produced an average glycemia of 0.1370 per cent. The average increase the first day was 0.028 per cent and the average increase the next day was 0.0366 per cent. There was apparently an increased tendency for ether to liberate dextrose the day following half an hour of ether anesthesia. The reason for this increase we do not attempt to give at this time. However, we are able to make the important deduction that ether did not injure the mechanism involved in the production of ether hyperglycemia. If such an injury had occurred the reaction to ether the second day would have been less than that of the first day and the normal glycemia before anesthesia the second day would have been less than that of the first day.

The effect of chloroform on the glycemia of the following day is shown in tables 3 and 4. The average blood sugars before ether of the first and second groups of animals on the day following the chloroform anesthesias were 0.096 and 0.0928 per cent respectively. These values compared with those of normal dogs shown in tables 1 and 2—0.1028 and 0.1004 per cent—indicate a decided tendency of chloroform to partially paralyze the mechanism of sugar mobilization. This phase of the action of chloroform is of great importance. In view of the conclusion of Cannon that blood sugar is the most satisfactory source of energy in emergency, it is of the greatest importance that the mobilization of blood dextrose be not interfered with at a time such as that following an operation when often every vital function is strained to the limit in order to sustain life.

Fifteen minutes of ether anesthesia the day following a chloroform anesthesia of half an hour produced an average glycemia of 0.1168 per cent for the group of non-fasting dogs and a glycemia of 0.1112 per cent for the group of fasting animals. These glycemias compared with those of the group of normal dogs (0.1308 per cent) and of the group which had an ether anesthesia of half an hour the preceding day (0.1370 per cent), show a decided inability of the animals given chloroform the previous day to develop as great a hyperglycemia as normal animals.

The increases in blood sugar due to fifteen minutes of ether anesthesia of the groups of animals which received a chloroform anesthesia the preceding day, were 0.0208 and 0.0184 per cent. The average increase due to ether anesthesia of untreated dogs given in table 1 was 0.028 per cent and the average increase of a group of 17 dogs, given in a previous publication (4), was 0.037 per cent. A comparison of these increases from ether anesthesia obtained the day following a chloroform anesthesia with the increases of normal dogs shows a considerable decrease in the power of the animals to mobilize dextrose after chloroform anesthesia.

There were two groups of animals that were given ether the day following chloroform anesthesia of half an hour. These animals differed in that the second group fasted two days before the tests were made. The day following chloroform anesthesia the non-fasting animals had an average glycemia of 0.0960 per cent and the fasted animals a glycemia of 0.0928 per cent. Fifteen minutes of ether anesthesia of unfasted dogs produced an average increase of blood dextrose of 0.0208 per cent, making a glycemia of 0.1168 per cent. A similar ether anesthesia of the fasting animals produced an increase of 0.0184 per cent making the average blood dextrose value 0.1112 per cent. A comparison of the normal glycemias, the increases due to fifteen minutes of ether anesthesia, and the glycemias after the ether anesthesia of the group of fasting animals and of the group of non-fasting animals, shows greater values for blood dextrose in every case for the non-fasting group. These differences are small but constantly in favor of the one group.

SUMMARY AND CONCLUSIONS

A group of dogs was anesthetized with ether for half an hour. The next day the dogs were anesthetized with the same drug for fifteen minutes. The blood sugar changes were measured for the first fifteen minutes of anesthesia both times.

A second group of dogs was anesthetized with chloroform for half an hour and the following day each was given fifteen minutes of ether anesthesia. The blood dextrose changes the second day were measured.

A third group of animals fasted two days and was then treated the same as the second group.

Half an hour of ether anesthesia did not alter the glycemia of the following day, and did not decrease the hyperglycemia resulting from fifteen minutes of ether anesthesia.

Half an hour of chloroform anesthesia produced on the following day a glycemia lower than normal, an increase in blood dextrose due to fifteen minutes of anesthesia less than normal, and a hyperglycemia from fifteen minutes of ether anesthesia lower than normal.

A fast of two days preceding half an hour of chloroform anesthesia produced on the following day a still lower glycemia and still less reaction to fifteen minutes of ether anesthesia than occurred in non-fasting dogs.

These results in conjunction with the conclusions of Davis and Whipple (2) that the liver injury produced by chloroform is increased by a fast preceding anesthesia leads us to the following conclusions:

1. Ether anesthesia does not produce any injury to the mechanism of dextrose mobilization that can be detected the following day.
2. The injury to the liver cells produced by chloroform anesthesia reduces the glycemia of the following day and injures the mechanism of dextrose mobilization according to the degree of injury.
3. The hyperglycemia due to chloroform anesthesia is not due primarily to the direct action of chloroform on the liver. Probably chloroform, like ether, produces hyperglycemia chiefly through its depressing action on the internal secretion of the pancreas.

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DIGESTIBILITY OF SOME HYDROGENATED OILS[1]

ARTHUR D. HOLMES AND HARRY J. DEUEL, Jr.

From the Office of Home Economics, U. S. Department of Agriculture[2]

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Until quite recent times table and culinary fats used in the United States were obtained almost wholly from the animal kingdom—dairy butter being the universal table fat and lard the principal culinary fat. The constantly decreasing per capita supply of animal fats has caused a very rapid increase in the use of vegetable oils for food, until olive, cottonseed, peanut and corn oils are now more or less generally used not only for salad but also for cooking purposes. Except for salad purposes, in the past the housewife apparently preferred fats which were very nearly, if not actually, solid at ordinary temperatures. To meet this demand for solid fats, vegetable oils were hardened either by removing a portion of their low melting constituents or by the addition of stearin or a fat rich in stearin, and fats prepared by one or the other of these methods came into quite general use under a variety of trade names. These processes for hardening vegetable oils have now been largely replaced by the hydrogenation process which is based on the discovery that hydrogen may be added, under proper conditions of temperature and pressure, to the glycerides of the unsaturated fatty acids by means of a catalytic agent, such as nickel in a finely divided state. When the process is carefully controlled it is possible to prepare hydrogenated oils having any desired melting point.

Since it is well known that some metals when taken into the alimentary tract under certain conditions are toxic, it has been very properly questioned whether the ingestion of hydrogenated oils containing appreciable amounts of a metallic catalyst might not be followed by harmful physiological disturbances. Recent investigations (1) indicate that properly prepared hydrogenated oils do not contain sufficient nickel to produce toxic effects. The Federal Meat Inspection Division (2) shares this opinion and permits the sale for edible purposes of those hardened oils which do not contain over two parts of the catalyzer per million parts of oil—an amount about five times that ordinarily found in normal hydrogenated oils produced in this country.

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[1] Published with permission of the Secretary of Agriculture.

[2] Prepared under the direction of C. F. Langworthy, Chief, Office of Home Economics.

The literature contains relatively little information regarding the digestibility of hydrogenated oils. Thoms and Muller (3) found that a hardened oil melting below body temperature was more satisfactory than one in which more complete saturation occurred and a harder fat obtained. Smith, Miller and Hawk (4) determined the relative digestibility of lard (melting at 45°C.) and hydrogenated cottonseed oil (melting at 36°C.) and found the lard to be 94.7 per cent and the hardened oil 93.4 per cent digested—a difference, which in the opinion of the authors, is well within the limits of experimental error. Pekelharing and Schut (5) found that on a diet which contained no fat other than hydrogenated cottonseed oil, mice maintained a steady body increment. They also determined the digestibility of some hydrogenated cottonseed oils in feeding experiments on a dog. On the average the dog digested about 90 per cent of the hydrogenated oils and increased its weight by about one-third the original weight. From the results of the four months' experiment they found that the digestibility of the hydrogenated oils was inversely proportional to their melting points, that mixtures of lard and hardened oils were more completely digested than the hardened oils alone, that no physiological disturbances occurred, and that the feces of the hydrogenated oil diets contained more fat and more fatty acids than those resulting from the lard diet. Erlandsen, Fridricia and Elgstrom (6) report studies of hardened whale oil in which the digestibility varied from 91.6 per cent to 94.9 per cent for butter and whale oil; the difference in digestibility of the two fats did not exceed 0.9 per cent.

The present paper reports a series of digestion experiments with hydrogenated cottonseed, peanut and corn oils in which the entire sample was subjected to the hydrogenation process. Material is also available for reporting the results of experiments on the digestibility of blended hydrogenated oils, in which some hydrogenated oil, hardened to a high melting point, was mixed with enough untreated oil to give a fat of the desired hardness. This investigation is a continuation of an extended series of digestion experiments which has been conducted by this office with about fifty more or less common animal and vegetable fats. The general conclusions from these studies are that edible fats are highly digestible, that they do not unfavorably influence the digestibility of other constituents of the diet, and that when eaten in normal amounts they do not cause any pronounced laxative effects.

Experimental. In these experiments the hydrogenated oils under consideration were included in the diet by being incorporated in the usual specially prepared cornstarch blancmange (7) or pudding. The basal ration, which was nearly fat-free, consisted of wheat biscuit, fruit, sugar and clear tea or coffee. The subjects of the experiments reported below were young men, apparently in normal health, from 20 to 40 years old, students in local universities, who as a result of their previous experience in this type of studies were familiar with the experimental procedure and who were thoroughly trustworthy. The experimental methods for separation and collection of feces and analysis of foods and feces outlined in earlier papers (8) were followed in the experiments below.

The presence of ether-soluble metabolic products in the feces has been taken into account and a suitable correction introduced in calculating results. Since the feces of a given day do not necessarily represent the residue of the food for the preceding day, it has seemed best in these experiments as in all the preceding ones to identify the feces of the experimental period with charcoal or carmine markers and to retain all the feces belonging to the period for analysis. Since the experimental procedure has been uniform throughout the tests on the animal and vegetable fats, the results obtained in these experiments are directly comparable with each other and with the results of our earlier studies.

Results of studies of the digestibility of common fats and oils indicate that their digestibility varies inversely with their melting points in the case of those melting above body temperature. In order to study the relationship between the digestibility and melting points of hydrogenated oils, those considered here were so chosen that some melted above and some below the temperature of the body (37°C.).

The majority of the samples of hydrogenated oil were hardened in the laboratory of Carleton Ellis by one of us (H. J. D.). J. R. Kuhn, of that laboratory, assisted in their preparation.

The melting points of the hydrogenated cottonseed oil were 35°C., 38.6°C. and 46°C.; the melting points of hydrogenated peanut oil were 37°C., 39°C., 43°C., 50°C. and 52.4°C.; and the melting points of hydrogenated corn oil were 33°C., 43°C. and 50°C. The iodine numbers of the samples are given in table 4.

All the hydrogenated oils included in this study were of a white color, solid or practically so at ordinary room temperature, and without any characteristic odor or flavor. When melted they were of a straw yellow color resembling melted tallow. They were very homogeneous and if heated sufficiently they boiled without any sputtering and did not smoke until a relatively high temperature was reached. On cooling, in some instances, different portions of the resulting mass differed slightly in physical appearance, quite likely because of a partial separation of the softer and harder constituents of the hydrogenated oils.

The experiments made with the hydrogenated corn, cottonseed and peanut oils were carried out under conditions essentially the same as those of experiments with the same kinds of oil untreated. As earlier reports show, those oils had coefficients of digestibility as follows: corn oil (9), 96.9 per cent; cottonseed oil (10), 97.8 per cent and peanut oil (11), 98.3 per cent.

Digestibility of hydrogenated corn oil. Fifteen digestion experiments were conducted with hydrogenated corn oil, five each with hardened fats having a melting point of 33°C., 43°C. and 50°C. These fats were prepared by one of the authors (H. J. D.) at a large commercial research laboratory and are believed to be typical of commercial hardened corn oil.

The same group of subjects assisted in each series of experiments with hydrogenated corn oils and the usual standardized experimental conditions were employed.

This report of the individual experiments with hydrogenated corn oil and other oils included in this investigation is somewhat condensed, but the experimental data in full are on file in the Office of Home Economics, States Relations Service, U. S. Department of Agriculture.

The data which were obtained from the study of hydrogenated corn oils are summarized in table 1.

The average amount of hydrogenated corn oil eaten per man per day was 78 grams for the fat melting at 33°C., 74 grams for the 43°C. fat, and 44 grams for the 50°C. fat. The digestibility of the hardened corn oils studied was for 33°C. oil, 94.7 per cent and for 43°C. oil, 95.4 per cent; thus from the standpoint of practical dietetics there was no material difference in the digestibility of these oils. The digestibility of hydrogenated corn oil melting at 50°C. was 88.5 per cent which is identical with that of mutton fat (12) (88 per cent) melting at 50°C. The coefficients of digestibility obtained in these experiments are somewhat lower than 96.8 per cent (13) reported in an earlier paper for the digestibility of commercial, edible corn oil.

The experimental diet as a whole was well utilized, the carbohydrates being very completely absorbed, which would indicate that hydrogenated corn oils having melting points a very little higher than body temperature did not have any unfavorable effect on the digestibility of the other constituents of the diet.

TABLE 1

Summary of digestion experiments with hydrogenated corn oil in a simple mixed diet

EXPERIMENT NUMBER	MELTING POINT OF HYDROGENATED CORN OIL	SUBJECT	DIGESTIBILITY OF ENTIRE RATION			DIGESTIBILITY OF HYDROGENATED CORN OIL
			Protein	Fat	Carbohydrate	
	°C.		per cent	per cent	per cent	per cent
1022	33	W. V. D.	75.3	90.8	97.8	93.5
1023	33	H. L. G.	71.0	93.6	96.8	96.7
1024	33	E. L. M.	69.6	91.5	97.4	94.5
1025	33	G. S. M.	72.2	87.6	98.1	90.7
1026	33	J. C. W.	72.0	95.0	96.9	97.9
Average			72.0	91.7	97.4	94.7
1037	42	W. V. D.	83.0	92.5	98.3	95.0
1038	42	H. L. G.	81.0	93.8	97.1	96.8
1039	42	E. L. M.	70.3	89.4	96.6	93.6
1040	42	G. S. M.	70.5	92.5	97.3	96.9
1041	42	J. C. W.	76.6	90.7	95.9	94.6
Average			76.3	91.8	97.0	95.4
1042	50	W. V. D.	65.2	84.8	97.8	90.2
1043	50	H. L. G.	65.9	81.6	95.9	87.9
1044	50	E. L. M.	86.2	91.2	98.3	94.0
1045	50	G. S. M.	60.7	78.9	97.5	85.6
1046	50	J. C. W.	69.9	79.4	97.0	84.9
Average			69.6	83.2	97.3	88.5

Digestibility of hydrogenated cottonseed oils. The experiments made with hydrogenated cottonseed oil were conducted under experimental conditions identical with those employed in the study of the digestibility of cottonseed oil which was found to be 97.8 per cent digested (14). The hydrogenated oils which have received attention in this investigation were not all prepared from the same lot of cottonseed oil; one lot melting at 35°C. (used in experiment 512) was a well-known commercial product which was purchased in the open market; the fat melting at 38.6°C. was specially prepared for our studies in the research laboratories of a concern manufacturing edible hydrogenated oils; the fats melting at 35°C. (used in experiments 1027–1031) and 46°C. were prepared by one of us in a large consulting laboratory. While these products were not all of commercial origin it is believed that they are, nevertheless, typical of the commercial article.

Table 2 summarizes the data from the experiments with hydrogenated cottonseed oil.

TABLE 2

Summary of digestion experiments with hydrogenated cottonseed oil in a simple mixed diet

EXPERIMENT NUMBER	MELTING POINT OF HYDROGENATED COTTONSEED OIL	SUBJECT	DIGESTIBILITY OF ENTIRE RATION			DIGESTIBILITY OF HYDROGENATED COTTONSEED OIL
			Protein	Fat	Carbohydrate	
	°C.		*per cent*	*per cent*	*per cent*	*per cent*
512	35	P. K.	67.0	92.6	96.6	96.2
1027	35	W. V. D.	84.8	96.9	99.0	98.3
1028	35	H. L. G.	61.5	94.4	95.2	98.6
1029	35	E. L. M.	70.2	94.4	97.9	97.0
1031	35	J. C. W.	63.6	89.7	95.7	93.9
Average..........................			69.2	93.6	96.9	96.8
459	38.6	R. L. S.	69.5	92.7	97.3	95.5
1052	46	W. V. D.	75.9	93.8	98.4	96.3
1053	46	H. L. G.	69.5	93.2	96.7	96.4
1054	46	E. L. M.	68.7	91.0	97.7	91.9
Average..........................			71.7	92.7	97.6	94.9

The average digestibility of the entire ration in the above experiments indicates that this diet was quite well digested. The daily consumption of hydrogenated oil in these experiments was on the average about 84 grams. In the last group of experiments with hydrogenated cottonseed oil melting at 46°C., 89 grams of the fat were eaten daily without causing any physiological disturbances, which would indicate that the limit of tolerance for this fat was in excess of 89 grams. The coefficients of digestibility, 96.8 per cent for hydrogenated oil having a melting point of 35°C., 95.5 per cent for hydrogenated oil having a melting point of 38.6°C. and 94.9 per cent for hydrogenated oil having a melting point of 46°C., indicate that the hydrogenated cottonseed oils having these melting points are well utilized by the body.

Digestibility of hydrogenated peanut oil. Twenty digestion experiments were made with hydrogenated peanut oils having melting points of 37°C., 39°C., 43°C., 50°C. and 52.4°C. under the usual uniform experimental conditions. The peanut oils melting at 39°C. and 52.4°C. were prepared for us through the courtesy of the chief chemist of a large manufacturing concern. The oils melting at 37°C., 43°C. and 50°C. were hydrogenated by one of us at a commercial fat and oil research laboratory. The results of the experiments with hydrogenated peanut oil are given in table 3.

The average amounts of hydrogenated peanut oil eaten daily in the above groups of experiments were: 37°C. fat, 76 grams; 39°C. fat, 78 grams; 43°C. fat, 91 grams; 50°C. fat, 59 grams; and 52.4°C. fat, 62 grams. No instance of intestinal disturbance resulted which indicates that the above quantities of these hardened oils are well tolerated by the average adult.

A comparison of the melting points and digestibility of these hardened oils is of interest. An increase of 2 degrees in the melting point (from 37°C. to 39°C.) was accompanied by a 2 per cent decrease in digestibility, an increase of 4 degrees from 39°C. to 43°C. caused no significant change in digestibility, an increase of 7 degrees from 43°C. to 50°C. caused a decrease of 4.5 per cent in digestibility, while an increase of 2.4 degrees from 50°C. to 52.4°C. caused a decrease of 13 per cent in digestibility. The coefficient of digestibility of 79 per cent for peanut oil melting at 52.4°C. is the lowest value obtained for any fat of this series; the digestibility (15) of mutton fat (melting point 50°C.) being 88 per cent and that of oleo stearin (16), 80 per cent. Since the melting point, 52.4°C., of this hardened oil is considerably higher than 37°C., the temperature of the human body, it is probable that in the process of digestion saponification takes place only on the exterior of the particles of hardened oil which decrease in size as the process of digestion continues. If surface area be thus a factor, then the rate of digestion and possibly the extent of digestion of a hydrogenated fat having a high melting point is governed to some extent by the size of the particles of hydrogenated oil ingested. Additional experiments are necessary to supply conclusive evidence on this point.

The hydrogenated oil melting at 37°C. was as completely digested (98.1 per cent) as the untreated peanut oil which was found to be 98.3 per cent absorbed (17) by the body. As a group the hydrogenated peanut oils were well digested and well tolerated by the subjects of this investigation.

TABLE 3

Summary of digestion experiments with hydrogenated peanut oil in a simple mixed diet

EXPERIMENT NUMBER	MELTING POINT OF HYDROGENATED PEANUT OIL	SUBJECT	DIGESTIBILITY OF ENTIRE RATION			DIGESTIBILITY OF HYDROGENATED PEANUT OIL
			Protein	Fat	Carbohydrate	
	°C.		*per cent*	*per cent*	*per cent*	*per cent*
1012	37	W. V. D.	74.7	95.1	97.5	97.6
1013	37	H. L. G.	78.3	96.9	97.5	99.1
1014	37	E. L. M.	68.3	94.3	97.4	97.4
1015	37	G. S. M.	57.7	94.0	96.3	97.9
1016	37	J. C. W.	66.7	94.9	95.8	98.6
Average			69.1	95.0	96.9	98.1
464	39	H. R. G.	74.0	95.4	98.1	97.8
466	39	P. K.	81.3	94.2	97.8	96.3
467	39	R. L. S.	66.7	90.2	96.8	93.7
Average			74.0	93.3	97.6	95.9
1032	43	W. V. D.	87.0	95.9	98.3	97.6
1033	43	H. L. G.	71.0	93.7	95.0	97.5
1034	43	E. L. M.	74.6	91.1	97.2	94.1
1035	43	G. S. M.	51.6	90.1	96.8	94.7
1036	43	J. C. W.	84.6	96.5	96.9	98.6
Average			73.8	93.5	96.8	96.5
1057	50	W. V. D.	70.0	87.0	98.2	90.6
1058	50	H. L. G.	65.4	88.5	96.1	93.1
1059	50	E. L. M.	69.9	90.3	97.8	93.6
1060	50	G. S. M.	69.0	86.6	98.1	90.7
Average			68.6	88.1	97.6	92.0
472	52.4	D. D. G.	65.0	65.8	96.4	71.6
473	52.4	H. R. G.	61.0	73.7	96.3	79.5
475	52.4	P. K.	41.7	81.8	98.9	85.9
Average			55.9	73.8	97.2	79.0

SUMMARY

Three vegetable oils, cottonseed, peanut and corn, have been partially hydrogenated to obtain hardened oils having different melting points and their digestibility studied. The results are summarized in table 4.

With the exception of peanut oil melting at 52.4°C. and the corn oil melting at 50°C. all of the hydrogenated oils were 92.0 per cent or more digested. They did not cause any observed digestive disturbances nor did they decrease the digestibility of the experimental diet as a whole. In general, the results showed uniformly that as the melting point of the oil was increased the coefficient of digestibility was decreased. The coefficient of digestibility decreased at a much faster rate in the case of fats with melting points above 46°. The number of experiments conducted with the majority of the hydrogenated oils under consideration, it is believed, is sufficient to permit of fairly definite conclusions regarding the digestibility of each of the oils in question.

TABLE 4

Summary of digestion experiments with hydrogenated vegetable oils in a simple diet

KIND OF HYDROGENATED VEGETABLE OIL	MELTING POINT OF HYDROGENATED VEGETABLE OILS	IODINE NUMBER	NUMBER OF EXPERIMENTS	DIGESTIBILITY OF ENTIRE RATION			DIGESTIBILITY OF HYDROGENATED VEGETABLE OILS
				Protein	Fat	Carbohydrate	
	°C.			*per cent*	*per cent*	*per cent*	*per cent*
Cottonseed...	35	89.6	5	69.2	93.6	96.9	96.8
	38.6		1	69.5	92.7	97.3	95.5
	46	72.8	3	71.7	92.7	97.6	94.9
Peanut.......	37	81.3	5	69.1	95.0	96.9	98.1
	39		3	74.0	93.3	97.6	95.9
	43	78.8	5	73.8	93.5	96.8	96.5
	50	58.5	4	68.6	88.1	97.6	92.0
	52.4		3	55.9	73.8	97.2	79.0
Corn.........	33	89.0	5	72.0	91.7	97.4	94.7
	43	74.9	5	76.3	91.8	97.0	95.4
	50	55.4	5	69.6	83.2	97.3	88.5

The results of these experiments indicate that these hydrogenated oils are as well utilized as natural fats of corresponding melting points.

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